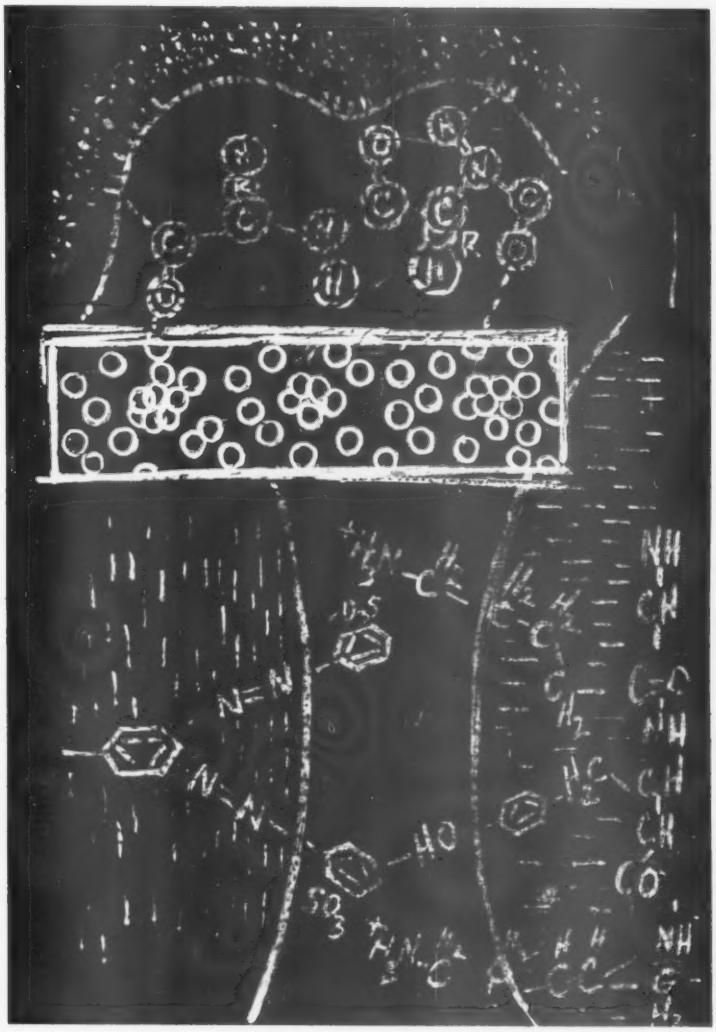
AGRICULTURAL

Science Review

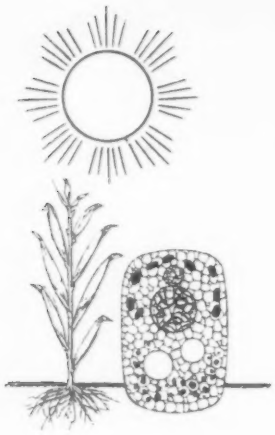
COOPERATIVE STATE RESEARCH SERVICE U.S. DEPARTMENT OF AGRICULTURE VOL. III, NO. 1



FIRST QUARTER

1965

ANTIGENS AND ANTIBODIES: THEIR USE IN STUDIES OF CELL DEVELOPMENT AND DIFFERENTIATION—PAGE 1



AGRICULTURAL SCIENCE REVIEW

First Quarter 1965 Vol. III No. 1

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EDITOR: WARD W. KONKLE

Chemicals in Agriculture

If this well-fed continent were called on to support its people with the tools of a century ago, we could very well rival some of the world's most underdeveloped nations in poverty and human misery. Of course that is an unlikely sort of "if." But there are around us the partisans of a chemical-free world who would have us give up some of the principal tools of modern agriculture.

The truth is that we have long passed that point where anyone can seriously think of going back to a chemical-free, technology-free agriculture. It is simply a matter of people to feed and clothe.

Our only hope for meeting the challenge of feeding a hungry world is a food explosion. Fortunately, this is possible. In the Western World we have the technology, equipment, and capital to increase world production of food tenfold in the next 10 years. But to accomplish it we will need to rely more than ever on the tools of our technology—fertilizers, pesticides, herbicides, growth regulators, defoliaters, and rodenticides. These tools are as indispensable as tractors, hybrid seeds, and improved crop varieties.

What lies ahead, therefore, is a high-density agriculture that will create an even more favorable environment for pests. To cope with this situation we will need more sophisticated, more selective, and more effective chemicals. New lands must be made fit for human life. More foods must be preserved, stored, and moved long distances and this means more and better antispoilage chemicals.

Amidst this aura of new technology, we must recognize the need to learn still more about all chemicals—the natural ones of our foods and those we formulate by necessity. Whether we like it or not, the world is becoming more crowded. But if we understand the chemistry of all things around us, we can have a more populous world and yet a safer and better nourished one than we have today.

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AGRICULTURAL SCIENCE REVIEW is published quarterly by the Cooperative State Research Service, U.S. Department of Agriculture, Washington, D.C., 20250.

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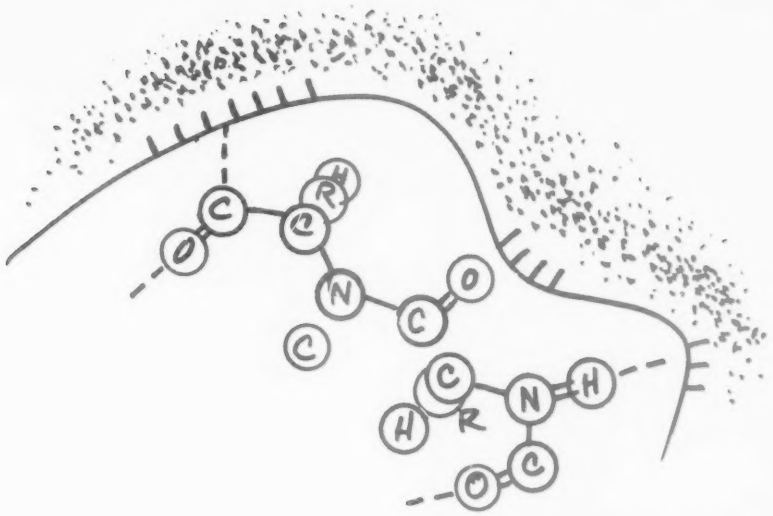

Antigens and Antibodies

Their Use in Studies of Cell Development and Differentiation

S. L. Scheinberg

How cells develop and differentiate has been of primary interest to biologists for over a century. It has been a challenging but perplexing area in which to conduct research. Only the most resolute have managed to maintain their interest and make contributions in spite of the enormous complexity of the material they worked with. The revolutionary advances in our knowledge of cell heredity in the last 10 years have now made it possible to attack the problems in these areas with renewed vigor. Opportunities for new advances come from a variety of fields and many disciplines. Studies in immunogenetics and immunochemistry offer many possibilities for studying the processes of cell development and differentiation. The use of enzyme antigens has led to very fruitful explorations with micro organisms and has increased our knowledge of the translation and coding process in cells. One of the model areas is the study of the structure and synthesis of antibodies with its potential application for the control of disease in humans and domestic animals. This report describes these and other studies with antigens and antibodies.

ANTIBODY STRUCTURE

In 1959 Lederberg (*21*)[1] outlined in very simple fashion propositions that would govern the structure and synthesis of antibodies. These propositions are being actively tested today. It is too early to determine if any of these propositions either in whole or in part are correct. The paper was important not only for providing a framework for experimentation but because it clearly included antibody synthesis as a cellular function governed by the same sequence of events which are responsible for the synthesis of other cellular proteins. Indirectly, primary attention was focused on the antibody, whereas most previous studies had emphasized the role of the antigen in evoking an antibody response. While antibodies are not homogeneous, they are all, nevertheless, globulin proteins, whereas antigens include every class of biochemicals from carbohydrates to

[1] Italic numbers in parentheses refer to "Literature Cited," p. 7.

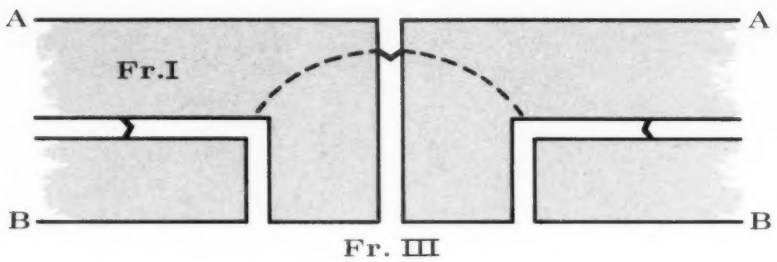

Fig. 1. Model of 7S immunoglobulin

1. **Signifies disulfide linkages.**
2. **Dashed areas indicate fragments obtained by treatment with papain activated with mercaptoethanol, i.e., Fr. 3 within dashed lines; Fr. 1 equivalent to Fr. 2 outside of dashed lines.**
3. **The overall dimensions are 240Å (long) × 57Å (high) × 19Å (thick).**

lipoproteins. An important aspect of this change in attitude was that the ever-present ambiguity of defining antigens in terms of antibodies or antibodies in terms of antigens could be resolved by regarding antibodies as a special class of globulins and operationally searching for serological specificity in the antibody structure.

The first proposition (*21*) is that the stereospecific segment of each antibody globulin is determined by a unique sequence of amino acids. The expectation that the serological specificity of the antibody—that is, the particular portion of the antibody molecule which combines with the antigen—is due to a particular sequence of amino acids conforms with the currently popular view in molecular biology that the amino acid sequence is the important facet of protein structure. Variations in amino acid sequence possess the potential for an almost infinite variety of antibody molecules—a very satisfying condition because of the well-known heterogeneity of antibodies.

The 7S Model

A great deal of information now available on the general structure of globulins has led to the formulation of some detailed working hypotheses. A number of models have been proposed for the general structure of the class of immunoglobulins designated 7S (*6, 22, 9*). The pertinent properties of these models have been combined and are shown in figure 1. The primary features of the model are the four polypeptide chains—two A and two B—with A chains joined to the B chains by disulfide linkages as indicated. In one model (*6*), both A and B chains are involved in combination with the antigen; the site is depicted at the ends of the molecule. According to another model (*9, 10*), however, only the A chains of horse gamma globulin possess antibody activity. Evidence from a variety of antigenic systems and with antibodies from many species will be necessary before this question is resolved.

The evidence for the model derives from numerous cleavage experiments. The now classical results (*23*) showed that purified 7S gamma globulin could be cleaved with the mercaptoethanol-activated papain which results in three fragments, of which I and II are indistinguishable (fig. 1). Fragments I and II possess serological activity, but fragment III does not (table 1). The 7S molecule could be separated as half molecules by mild reduction with mercaptoethanol followed by treatment with hydrochloric acid. Reduction of interchain disulfide bonds in the absence of urea followed by exposure to urea or acid solutions leads to dissociation of the A from the B chains (*6*). The separated A and B chains will reassociate to form 7S molecules with molecular weights of 160,000. If the chains are in the partially reduced state, the

interchain disulfide bonds can be reformed in a chain arrangement grossly similar to that of native γ-globulin. A and B chains of a variety of types and even of different species will interact to form 7S molecules, suggesting similarity of regions among these chains.

There is evidence that the chains have no α-helical structure, but secondary structure of segments of the chains in the β conformation has been suggested (*14*).

The relationship of 7S to 19S (the second major class of immunoglobulins according to molecular weight) is not entirely clear; available evidence suggests that 7S molecules are not simple subunits of the 19S molecule. Mild reduction results in the production of some subunits resembling 7S antibodies. Presumably, these fractions possess B chains in common with the 7S antibodies. However, there are differences in carbohydrate content, sedimentation velocity, and in the antigenic properties between the reduced fractions and the 7S immunoglobulin (*4*).

The Antibody Site

Numerous reports have indicated the heterogeneity present in antibody solutions (*6, 34*). Such heterogeneity reflects variation in size of immunoglobulins, a subject already alluded to. It is also a response to the multiple specificities possessed by most antigens used in immunization, and it reflects the capacity of immunoglobulins to act as antigens. Physical properties attributed to the globulin because of their antigenic features have been confused with those due to the antibody itself. There is a growing body of evidence that amino acid composition and, more likely, amino acid sequence are involved in antibody specificity. The difficulties attending the purification of antibody molecules or substructures have impeded progress in this area but, in spite of the problems, the studies continue.

A number of studies clearly demonstrate that B chains of guinea pig antibodies may be separated on starch gel electrophoresis, which suggests that these chains differed in charge and presumably in amino acid composition or sequence. These dif-

TABLE 1.—ANTIBODY FRACTIONS

Designation	Sedimentation coefficients	Molecular weight	Nomenclature		Additional designations
			Antibody activity	Carbohydrate content	
γ-globulin	6.5S, 7S	150, 000	Yes	2–3%	
γ_{1A}	(predom.) 7S–13S*		Yes	9%	B_2A
γ_{1M}	19S	750, 000–850, 000	Yes	10%	B_2M
γ-microglobulins	2.35S		No	0%	
B chains		20, 000	Yes (2); (4)		L
A chains		53, 000	Yes (2); (4)		H
Fragment I or II		45, 000	Yes		S (slow)
Fragment III		55, 000	No		F (fast)
Bence-Jones proteins (L-chain like)		20, 000	Yes		

ferences are associated with antibody specificity or alternatively with the nonantibody segment of the chain (*5*). Other studies (*13, 12*) have shown that for individual haptens there were amino acids which appeared to be important. The combining sites of rabbit antibodies specific for the haptens p-azobenzenearsonate, p-azobenzoate, p-azophenyltrimethylammonium, and 3-azophyridine groups are partially inactivated by treatment with iodine or acetic anhydride. In each treatment the presence of a specific hapten gave some protection. The activity lost during acetylation could be partially restored by treatment with hydroxylamine which indicates that the effect of acetic anhydride came at least in part from esterification of hydroxyl groups. These results and others are consistent with the presence of a tyrosine side chain in the active sites of at least part of each of these antibody populations.

A vigorous attempt has been made (*19*) to detect differences in amino acids among antibodies and to avoid the ambiguity due to the globulin molecule's antigenic property. Rabbit antiserum was prepared against the haptens, phenyltrimethylammonium and phenylarsonate. The genetic differences in antigenic properties of the globulins (allotypy) in the rabbits was determined. The experiments were then conducted with individual rabbits so that these antigenic properties would not interfere in the analyses. Elaborate procedures were carried out to insure the purity and homogeneity of the antibody solutions and then amino acid determinations were made of the hydrolysates. Antibodies directed against the phenyltrimethylammonium hapten possessed a larger number of leucine and aspartic acid residues, whereas the antibody directed against the phenylarsonate hapten possessed more arginine and isoleucine. The differences between the two antibody types were two to four residues per molecule; the results were significant and reproducible. In spite of all the precautions taken, there was the possibility that the amino acid differences were due to the sampling from a pool of heterogeneous antibody molecules, the heterogeneity being due to antigenic as well as antibody differences among the globulin molecules of a given rabbit.

A repetition of the experiments was carried out with even greater care with individual rabbits known to be heterozygous or homozygous for allotype and which were injected with protein to which both haptens were conjugated. It was clearly shown that there were amino acid differences in the antibodies directed against a third neutral hapten (p-azophenylactoside) as opposed to that of the others. Antibodies against this hapten contained more aspartic acid, whereas antibodies against p-azobenzenearsonic acid contained more tyrosine (*20*). These experiments provide additional evidence that amino acid composition, if not sequence, determines the site.

An unresolved question is the type of bonding in the antigen-antibody reaction. It has been suggested (*19, 17*) that the bonding between antigen and antibody is covalent. The evidence indicates that charged haptens such as phenyltrimethylammonium evoke antibodies with a greater number of amino acids of opposite charge; i.e., aspartic acid (*19*). It is also clear from these studies (*20*) that a neutral hapten (p-azophenylactoside) may stimulate antibodies which possess an unusual number of charged residues (aspartic acid) (*20*). There is also evidence for noncovalent bonding, such as hydrogen bonding.

Experiments have been carried out with human A red cell stroma to which anti-A antibodies have been complexed (*34*). It is possible to dissociate these complexes by thermal means and moreover to obtain distinct antibody populations which dissociate predominantly at either 37°C, 45°C, or 56° C—the temperatures used in the experiments. Evidence from other thermal dissociation experiments (*17*) also supports noncovalent bonding between antigen and antibody.

At the antibody specific site, both covalent and noncovalent bonding may exist. A model for such a combined bond is suggested by the technique of affinity bonding (*33*) which provides for a covalent bond in addition to the bond between the antibody and the antigen. In one such experiment, the reagent p-(arsonic acid) benzenediazonium fluoroborate was used to label the active site of specifically purified anti-p-azobenzenearsonate antibody. The benzenearsonate portion of the molecule is responsible for combining with the hapten, and the diazonium group appears to act preferentially with a side chain at the site.

Lectins or plant hemagglutinins are increasingly being used in blood grouping, and the study of the properties and structure of lectins is an important

facet of the study of antibodies (*34, 31*). It is to be expected that lectins for human A cells, for example, will possess the same or similar structure at the antibody site as those of antibodies in blood and therefore should constitute an important test of the detected structure.

Whatever the nature of the bonding may turn out to be, the ultimate resolution of the antibody structure and of the nature of the antibody combining site itself appears excitingly close.

ANTIBODY SYNTHESIS

While there has been substantial progress in the determination of the structure of antibodies, only a start has been made in the study of antibody synthesis *in vivo*. A few of the steps are known but only in the crudest sense. Curiously, for immunology, theory has outpaced the level of experimental knowledge. The major difficulties are technical with problems encountered in the growth and manipulation of cells in culture. Currently, elements of the elective or similar hypothesis of antibody formation are being tested (*15, 3*). In this hypothesis a heterogeneous population of globulin-producing cells causes the production of a heterogeneous population of globulin molecules. The variation in cells is due to mutation and selection. The selection is made possible by the introduction of an antigen which favors clones of cells with antibodies directed against it. This hypothesis was an attempt to account for at least three immunological phenomena. First, the now well-known cellular stimulus following antigen injection; second, immunological tolerance (inability of an organism to produce antibodies against its own tissues or those introduced at a very early period in its growth); and third, the anamnestic response—a marked immunological response occurring after an initial stimulus and usually after an interval of time; i.e., months and even years intervening between the initial and final injections. The hypothesis was satisfying in many respects and provided a rational scheme, particularly for tolerance, which alternative hypotheses failed to do. The instructive hypothesis (the major alternative) considered the stimulus by the antigen as the primary step. The antibodies are formed or molded following the introduction of antigen in the cell and continuous production requiring a residue of antigen.

The requirement that antigen always be present to promote antibody synthesis regardless of the time interval is a difficult point to establish experimentally. However, there are instances in which antibody has been known to persist for long periods of time (*11*). Even more difficult to determine are the crucial events in the fate of antigens that lead to the induction of antibody formation. On the other hand, the most difficult part of the elective hypothesis is the requirement that all cells be preadaptive. This would mean that cells possess globulins for every antigen with which they will ever come in contact. In all likelihood elements of both hypotheses will be found essential for the synthesis of antibodies.

A number of very interesting experiments are beginning to provide the experimental framework for rigorous tests of the hypothesis. Experiments have been conducted on *in vitro* production of antibody formation with bacteriophage T_2 as antigen (*7, 8*). It was found that macrophages are the first cells to come in contact with antigen; RNA from these cells, with or without antigen, can be transmitted to active lymph node cells in diffusion chambers placed in the peritoneal cavities of rats. Sera from such rats were assayed and found to possess phage-inactivating sera. Verification with other antigens and other organisms should indicate the general validity of these findings. It is an area of great challenge and of profound significance.

ANTIGENS AS MARKERS

Micro-organisms

The potential contribution to our understanding of cellular control by the continued study of antibody synthesis appears to be great. The use of antigen markers in studies of cellular control has been more limited but appears to possess equal, if not greater, potential. This has been demonstrated in a very elegant fashion with the analysis of tryptophan synthetase (*35*). The reactions in which this enzyme plays a role are the steps from anthranilic acid to tryptophan, in which tryptophan synthetase catalyzes the reactions: (*a*) indole glycerol phosphate to indole and tryptophan, and (*b*) indole to tryptophan with the major pathway being indole glycerol phosphate to tryptophan.

anthranilic acid→indole glycerol
phosphate→tryptophan
⇵ ↗
indole

The enzyme tryptophan synthetase includes two proteins, A and B, both of which are necessary for catalytic steps from indole or indole-glycerol phosphate to tryptophan. Antisera have been used to distinguish the A from the B components and to indicate similarities and differences between proteins from mutant strains and the wild type in both *E. coli* and Neurospora. It has also been possible to use antibody solutions to inhibit the activity of various tryptophan synthetase proteins. Serological specificity as well as suppressor mutations have been used to characterize and map the tryptophan synthetase mutants. More recently, the antigenic properties of the enzyme have been separated from that of the enzyme activity itself (*16*). Additional studies have led to the determination of specific amino acid substitutions at different locations in the tryptophan synthetase proteins and to demonstrate colinearity of amino acid sequence with that of the genetic map (*36*).

Vertebrates

The use of micro-organisms has made it possible to carry out many experiments, particularly at the molecular level. This situation is likely to prevail for some time and there may even be an expansion in the use of micro-organisms. Nevertheless, serious attempts are being made to conduct experiments with higher organisms. In these studies, the use of antigen markers promises to provide the means for detailed cellular studies.

There is now abundant evidence that somatic cells of a given tissue are not homogeneous and undergo considerable changes during growth and development (*32, 18*). An example of such heterogeneity is that associated with the red cell antigen A_1.

It has been found that all human A and pigeon A bloods examined possess a small fraction of cells which lack the antigen possessed by the major cell population (*1, 27*). An isotope dilution method showed that the frequency of these exceptional cells ranges between $1\times10^{-2}-1\times10^{-4}$. Moreover, it is possible to increase the frequency of these cells by irradiation (*27*). Additional studies are in progress to determine the origin of the exceptional cell.

One of the most interesting recent examples of cellular heterogeneity is the apparent lack of participation in cell function by one of the x-chromosomes in human females. As a result of such nonparticipation or x-chromosome inactivation, women heterozygous for the enzyme glucose 6-phosphate dehydrogenase possess two types of red cells—those producing a normal amount of the enzyme and cells with a deficiency of the enzyme (*2*). It appears to be entirely a matter of chance whether the maternal or paternal x-chromosome is inactivated.

The Barr body, a drumstick-shaped mass in the nucleus which is characteristic of the cells of females, is also presumably associated with the inactivation of one of the x-chromosomes. However, not all of the x-chromosome genes or gene products may be inactivated. The antigen X^{ga} which is produced by the action of a sex-linked gene does not appear to be affected, for mosaicism with respect to this antigen has not been found (*24*). However, there is need to establish that the X^{ga} antigen is a direct product of the cell nucleus.

Another example of somatic cell heterogeneity involves the cellular marker, "Hi." The "Hi" substance of chicken red cells was detected only in sexually mature females (*28, 29*) and associated with egg production. This association was due to one of the components of egg production, estrogen, and it provides a definitive example of relationship between a physiological trait and an agglutinogen at the cellular level. Such associations have been sought and continue to be looked for by many blood group workers in many areas (*25, 26*). It is now known that the "Hi" agglutinogen is under the control of the genotype as well as estrogen. The "Hi" substance requires the presence of a single autosomal dominant gene for its formation. Stimulation of genetically competent males or of young birds with estrogen results in the appearance of the "Hi" substance on their red cells as well.

Inhibition experiments with simple sugars indicate that D-mannose or a similar sugar is an important part of the agglutinogen and that the sites of attachment to the agglutinin (*Pisum arvense*) involves the C_4 and C_6 hydroxyl groups (*30*). The heterogeneity with respect to the "Hi" substance in the red cell populations of genetically competent birds is a function of the level of estrogen and the cell lifetime. In birds just beginning to lay or in-

jected with estrogen, the terminal red cells lack the "Hi" substance. These cells are replaced by cells possessing the "Hi" substance. In the converse situation when hens are going out of egg production or estrogen injections have ceased, "Hi" positive cells are replaced by negative cells. It is hoped that this particular model system will be of value in the study of cellular control. Material such as this and the other examples cited offer many opportunities for studies of population dynamics, growth, and development in higher organisms.

SUMMARY

Immunogenetic and immunochemical model systems will undoubtedly continue to contribute to our understanding of cell development and differentiation. Our knowledge of individual cellular processes in intellectual development in man or in aging of both man and other animals and the physiological processes in the production of milk, eggs, or wool will increase as our knowledge of cellular control increases.

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A Worthy Nutritional Project

Termination of Regional Project No. NE–37 has marked the end of a fruitful 8-year study on the metabolic relationships between protein and selected nutrients. Twelve States [1] participated in research studies which now provide a theoretical basis for planning the economic use of protein resources in feeding animals and man. The findings—that deficiencies of several B vitamins or of energy impaired the utilization of protein—indicate a need for balancing other nutrients in the diet for the most efficient use of protein. On the other hand, observation that protein utilization is little affected by the proportions of the energy supplied by fat and carbohydrate indicates that the protein supply does not need to be adjusted when changes occur in the availability of fats and starches.

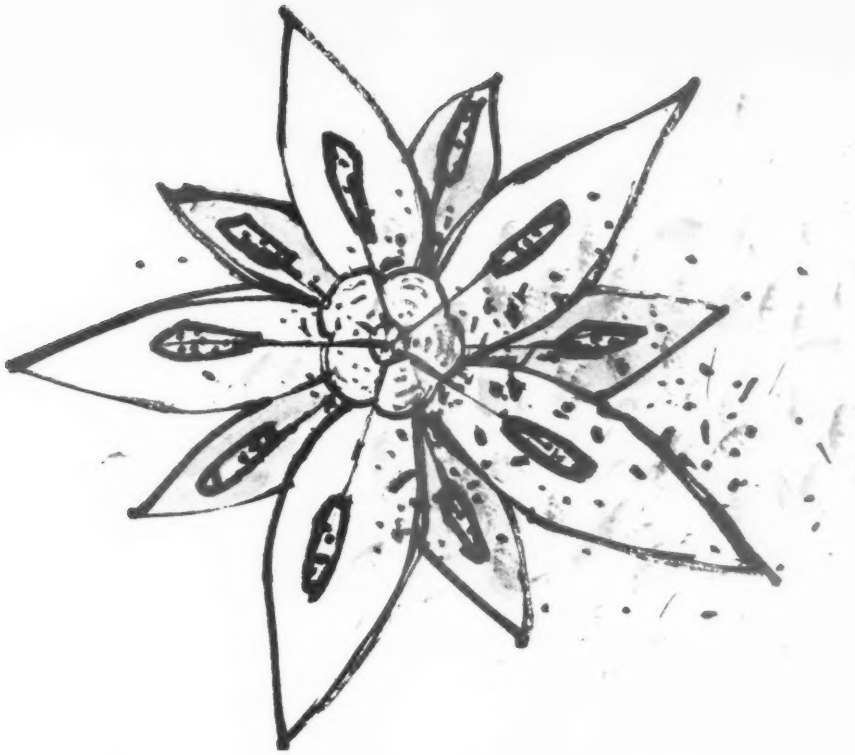
The finding that the amount of protein in the diet did not affect the levels of serum lipids in human subjects indicates that the protein level is not an important factor in the etiology of atherosclerosis in this country. However, the fact that supplementation with sulfur-containing amino acids did, under certain conditions, affect the serum lipid levels suggests caution in the use of amino acid supplements.

Many of the results from NE–37, such as relationships established between dietary nutrients and their metabolites in blood, tissues, and excreta, are significant in that when pieced with other research findings, they help provide an understanding of the basic processes of human metabolism. This understanding should lead to more efficient exploitation of our food resources.

This regional research has led to improvements in methods for (1) assessing nutritional status, (2) determining nutritional requirements, and (3) conducting nutritional studies of man under controlled conditions. Such improvements will also contribute indirectly to the nutritional knowledge that provides the basis for sound feeding practices.

During the 8-year period the project was active, participating States prepared 74 papers reporting various phases of the research work. Two were regional publications and 31 were graduate theses.

[1] Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, and West Virginia.



Physiology and Uses of Tree Pollen

Robert G. Stanley

Much agricultural industry is based on products of plant pollination. The production of practically all fruits and many vegetables and the maintenance and replacement of most range grasses and forest trees depend on pollen. A few species such as bananas, figs, seedless oranges, and grapes produce fruit without pollen. But generally, only fertilization by the male cell transmitted in pollen can produce viable seed. Understanding the physiological basis of pollen growth and how pollen can be manipulated to achieve desired results in breeding and crop production is a recent addition to scientific knowledge.

The Ancients recognized male and female trees. Dioeciousness occurs in certain species, including the date palm. About 4,000 years ago, the Egyptians selected male date palm branches and shook them over open flowers to assure a good fruit crop. Cave drawings and pyramid frescos show that the Egyptians also ate date pollen as a food delicacy.

Pollen was first germinated in solution in 1834. Not until 1884, however, was the process of fertilization in seed plants discovered. Pine and spruce trees provided the study material used to make the findings. In the last 80 years, considerable knowledge of pollen chemistry has been gained. This knowledge has made possible improved methods of collecting, storing, and using pollen. Increased ability to manipulate pollen has in turn led to improved fruit yield in agricultural crops and better trees for lumber and pulp in forestry.

Today, tree breeders or orchardists use pollen that has been collected, concentrated in a relatively pure form, stored, and then applied to the female flower during the short critical period of receptivity. The plant breeder is, therefore, concerned with purity of pollen and its ability to grow and to fertilize the egg. Procedures for collecting, storing, and

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testing have been developed for different pollens.

This paper describes the use of pollen, includes some of the more significant background knowledge of pollen physiology, outlines research in progress, and suggests the type of information needed to solve problems confronting tree breeders and orchardists.

POLLEN COLLECTION

How and when pollen should be collected depends on several factors. Among these factors are: species, intended use, growing condition of the plant providing the pollen, and the plant to which it will be applied. In fruit production, pollen is collected and applied to increase average fruit set in poor weather, or to insure high fruit yield. In fruit and forest tree improvement, pollen is used to produce hybrid offspring with desired growth characteristics.

Pollen collection begins with removal of either whole flowers or only the pollen-bearing anthers. One method, used with aspen or birch, is to collect branches a few days before flowers open. The branches with intact male flowers are placed in water. Pollen is shed and collected from around the container. An alternate method, used in pear or apple breeding, is to remove whole flowers when they are open but before the anthers have shed their pollen. These flowers are dried and rubbed on a fine screen sieve to free the pollen-filled anthers. Apricot or grape anthers are sometimes removed from fresh flowers and spread on a watch glass or glass dish; the anthers dry and release pollen, which is collected free of anther residue.

Variations in collecting procedure are adapted for particular flowers. In some flowers a sticky thread attaches pollen to the anthers. A vacuum collector has been adapted for such flowers. A comb or simple hand forceps have been used to collect pollen where anthers can be easily detached from the stamen. Pollen loads have been ingeniously scraped from bees by means of a collector attached to the hive entrance. The pollen mass removed from the bees is then used for pollination. Pine or birch pollen is collected by gathering catkin clusters which open on drying. Large amounts of genetically pure pollen are available from such trees.

The conditions under which pollen is handled and stored are critical factors in pollen survival. Under field conditions angiosperm pollen usually remains viable for a few hours or, at the most, a few days. Gymnosperm pollens, if away from direct sunlight, may remain viable several weeks. During drying or storage, pollen must be kept out of sunlight. Light of ultraviolet (UV) wavelengths decreases pollen ability to grow and set viable seed. It modifies the chemical compounds which control growth characteristics. To avoid this change, tree pollen is usually stored in airtight bottles away from light.

Most tree pollens survive better when stored at relative humidities between 5 and 10 percent; a few pollens require a critical moisture content above these levels (*7*).[1] Controlled moisture contents are obtained by storing the pollen over such desiccants as calcium chloride or sulfuric acid. Generally, dried pollen is stored in a refrigerator or deep freeze chest until used. Collection and storage of forest tree pollen is similar to that of fruit trees; however, variations in available quantity and length of storage life of pollens differ (*4*). For example, pine pollen can be collected in pound lots from a single tree and used 10 years later under proper storage conditions. Other pollens survive only a few days. Table 1 lists examples of survival of different pollens in storage.

In addition to temperature, moisture content, and ultraviolet radiation, pollen survival is also affected by exposure to gases and chemicals before and during storage. Temperatures as low as those of liquid nitrogen (−190° C.) do not adversely affect properly dried pollen. But few benefits would be gained by preserving pollen at such low temperatures. Storage at temperatures of −15° to −20° C., now available in home deep freezers, has become a common practice. This temperature can retard most cellular processes which lead to deterioration of stored pollen. Good results have also been obtained with pine and other pollens dried by freeze-evaporation (Lyophilization) and then stored at room temperatures (*2*).

The chemical composition of the pollen grain's outer layers helps explain why certain pollens can be stored for long periods. It has been postulated that chemical differences in the pollen outer coat (exine) and components in the inner membrane (intine), may influence survival. Pigments such as carotenes and flavones screen potentially damaging

[1] Italic numbers in parentheses refer to "Literature Cited," p. 17.

ultraviolet light. Changes in carbohydrate and organic acid content have been observed during storage. The levels of these compounds have been related to the capacity of pollen to germinate after storage. Increased organic acid and decreased sugar content accompany low pollen viability. While pollen ages during storage, viability can frequently be restored by exposure to a moist atmosphere for about an hour before germination is attempted. Increased sugar concentration is also frequently required for pollen germination *in vitro* as it ages in storage (*4*). These techniques reactivate germination of stored pollen. They indicate the nature of inherent chemical changes that adversely affect pollen.

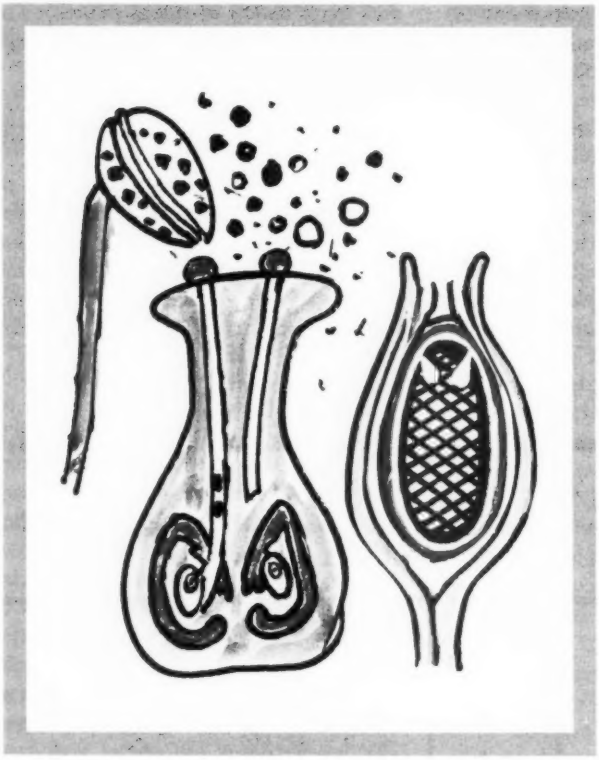
POLLINATION MECHANISMS

The probability that a single viable pollen grain will land on a receptive germination site in nature is infinitesimally small. Many species have developed the capacity to shed large numbers of pollen grains when female flowers are open. Others have evolved mechanisms for transporting pollen to

TABLE 1.—POLLEN STORAGE

Species	Days stored	Percent germination		Treatment and storage method [1]
		Initial	Final	
Pyrus communis	10	65	<5	R.T.
Pyrus communis	66	65	60	Freeze drying, R.T., N_2 gas
Pyrus communis	662	66	42	3° C., 10% R.H.
Pyrus communis	1,032	64	50	−20° C., vacuum
Pyrus communis	662	64	50	−190° C., vacuum
Pyrus communis	1,032	64	15	3° C., normal atmosphere
Citrus spp.	550	80	63	2° C., 25% R.H.
Hevea spp.	19	90	37	5° C., 75% R.H.
Betula lutea	30	40	3	R.T., 25% R.H.
Pinus taeda	30	80	<5	R.T.
Pinus taeda	86	80	79	Freeze drying, R.T., N_2 gas
Pinus taeda	379	80	32	Freeze drying, R.T., N_2 gas
Pinus ponderosa	1,100	90	80	4° C., 50% R.H.
Pinus ponderosa	2,160	90	36	4° C., 50% R.H.
Pinus ponderosa	2,160	90	3	4° C., 0% R.H.

[1] R.T. = Room temperature; R.H. = Relative humidity



the receptive female flower. Bees pollinate many fruit trees. Pollen is dislodged and picked up in the leg hairs of bees when they extract nectar from inside the flowers. It is then scraped off onto the stigmas of flowers they subsequently visit. To insure adequate fruit set, beehives are sometimes rented and set out in certain orchards or fields during flowering periods. If the weather is rainy, overcast, or cold, the bees will not function normally and low pollination and fruit set will result. At such times, or in place of bees, artificial pollination may be employed. Artificially controlled pollination is also commonly used to obtain hybrid offspring with certain desired growth characteristics.

In large orchards, a machine is often used to spread pollen about the tree. For tree breeding, pollen is generally applied to the flowers by hand with a fine hair brush, an air atomizer, a modified hypodermic syringe, or—to conserve pollen—a pencil eraser. If the species can be self-pollinated, an electric vibrator may be used to shake pollen onto the stigma.

If pollen is to be artificially applied, other pollen should be excluded from the flower. To prevent contamination, the pollinated flower or female cone is enclosed in a bag. These bags of coated plastic or specially fabricated paper are generally set in place before the flowers open. Selected pollen is sprayed into the sealed bags during the period of optimum receptivity. Such hand-pollinating methods are expensive, but they insure that only the desired offspring can arise if the pollen used germinates and fertilizes the egg cell.

In nature, wind-pollinated trees or insect-pollinated trees depend heavily on weather at flowering time. Low temperatures or rainy weather will retard flower opening and pollen shedding. The normal time is established by a genetically controlled physiological clock. Maturation cycles vary with species and frequently with subspecies. Occasionally, related species living close together flower at different times. During a year of inclem-

ent weather, their physiological maturation patterns may be abnormally modified. Thus, the normally early-flowering species may be delayed and shed pollen at the same time as a related species. Under these conditions crosspollination can occur and a hybrid may result. Such hybridization is common in species pollinated by windborne pollen, which is often carried for miles. Interspecific hybrids are common in cherries, pears, plums, and oaks (*1*).

Tree breeders often establish seed orchards to obtain seed of known genetic quality. This practice is necessary because of the delayed maturity and open-wind pollination pattern of many trees. Pines, like many other trees, may be 5 to 15 years old before they produce viable seed. Attempts have been made to induce early flowering and seed formation in such trees. The methods include selection, fertilizing, pruning of roots or tops, and subjecting the trees to modified light and temperature conditions (*10*). In seed orchards, the natural dispersal distance of pollen is important in prevention of pollination by undesirable trees outside the tree orchard. Control or elimination of stray pollen can, under such circumstances, be of great economic importance to the tree breeder or seed producer. To avoid the production of seed arising from pollen of unknown genetic quality, a seed production area or a seed orchard site must be very carefully isolated. Physical or chemical removal of potential outside pollen sources is usually required.

VIABILITY TESTS

If stored pollen is used in fruit or hybrid tree production, its ability to grow, fertilize the egg cell, and form seed must be determined before use. It is not always easy or possible to determine rapidly if pollen will give rise to good seed. In most pines, for example, seed is produced 2 years after pollination. Other trees require several weeks or months before seed is obtained. To avoid this long delay, a simple method of determining if stored pollen is alive and can germinate on the plant is desirable. Several laboratory tests for pollen viability have been developed. In one procedure, a dye is applied to the ungerminated pollen; in another, pollen is germinated in a chemical solution.

Chemical tests.—Viable pollen contains active enzymes that cause a color change in certain dyes when they are absorbed. These same enzymes are involved in pollen germination on the plant. The percent of colored grains in the dye test is assumed to indicate the percent that will germinate and fertilize the egg cell to form seed.

The pollen enzymes which react with the dyes depend on temperature and oxygen. Therefore, these tests are limited by temperature, oxygen concentration, and the ability of pollen to absorb the dye. Commercially used dyes are 2,4,5-triphenyl tetrazolium chloride and benzidine. Both chemicals form a red color complex in the presence of active oxidative or peroxidase enzymes. Such dye reactions are simple to use and require about 1 hour for results, but they are not valid for all species. Generally, dye reactions do not give reliable tests with old or poorly germinating pollen. If pollen grains turn red in a tetrazolium solution, this indicates only that active enzymes are present. The main drawback is that a positive color reaction is not always a valid basis for determining if pollen will give rise to viable seed. Enzymes present may react with the dye and yet not be active enough to support pollen tube growth to fertilization. One reason probably is that tube growth to the egg cell and nuclear division in the pollen tube to form the male cell represent a series of interrelated growth reactions. The dye reaction may merely mean that one enzyme in the growth reaction sequence is active.

Germination tests.—Viability assays which use pollen tube growth as indicators may be affected by many factors in the test solutions. The growing tube membrane consists primarily of cellulose and pectin. Thus, the factors involved in biosynthesis of tube membranes will regulate pollen growth. These factors include sugars, salts, and hydrogen ion concentration.

Sugars generally serve as a carbon source for germinating pollen, but not all sugars can be used. Furthermore, not all pollens require the same level of sugar in the germinating medium. For example, most pollen will not produce a tube unless sugars or minerals are present; pine pollen will produce a tube in distilled water.

Generally, 0.3 to 0.5 molar sucrose is used in germinating media. In the case of pear or apple pollen, raffinose is a better substrate than sucrose; glucose supports growth about equally as well as sucrose, and fructose is a poor substrate (*7*). Many

other sugars can also be used, in particular those which contain the α-1,4 glucose linkage common to sucrose and raffinose. The reason for this specificity is not known, but we assume that it is a function of pollen enzymes which limit metabolism of external sugars. We know that raffinose and sucrose, which support growth best in artificial media, are also present at fairly high levels in many female tissues that pollen must penetrate to reach the egg cell. We also know that pollen tubes secrete many enzymes necessary to metabolize starches and sugars outside the pollen. These enzymes are active and are secreted before pollen tubes appear (*12*).

GROWTH PHYSIOLOGY

Osmotic and chemical changes which occur in the stylar tissue where angiosperm pollen grows have been correlated with tube development. Tube formation is sensitive to osmotic pressure resulting from the sugar concentration around the pollen and the internal sugar content (*13*). Optimal osmotic concentration occurs at pollination time, while growth-inhibiting osmotic pressures decrease pollen enzyme activity (*12*). Ionic composition and con centration in the growth medium, either test solution or stylar pollen canal, also affect enzyme activity and pollen growth.

Between 1900 and 1930, many studies demonstrated that such minerals as sodium, potassium, and calcium affect pollen germination (*4*). Salt concentrations above 0.005 molar generally inhibit growth. Sodium is more inhibitory than potassium; calcium is the least inhibitory of all cations tested. In the early 1900's, it was also recognized that hydrogen ion concentrations are critical to pollen growth. Optimum pH varies with the species. Generally, the best growth occurs between pH 5.5 and 7.0. Stylar tissue pH has been correlated with the optimum pH determined in artificial growth media (*11*).

Other environmental factors also influence pollen growth. Optimum temperature for pollen grown in artificial media can frequently be related to temperatures occurring in nature at pollination time. Laboratory studies of pollen growth, however, are generally run at one temperature. In nature, diurnal temperature fluctuations may influence the rate of pollen tube growth to the egg cell. When the germination-to-fertilization process occurs within 1 hour, temperature variation is of little importance. But if fertilization occurs 24 hours or 1 year after pollination, temperature and surrounding microclimate, as well as intracellular chemical changes, can be critical.

The most dramatic intracellular chemical factor influencing pollen growth was discovered in 1932, when Schmucker observed that pollen grew better in stigma extracts than in sugar solutions. Analysis of the stigma fluid and pollen showed a high boron content. Adding borax and boric acid to a sugar solution markedly stimulated pollen tube growth. Studies throughout the world since Schmucker's discovery have shown that many pollens require boron for germination. The exact concentration

TABLE 2.—POLLEN TUBE GROWTH

Species	mm/hour	Style length (mm)
Malus domestica:		
(Rome Beauty × Jonathan)	0.35	7
(Rome Beauty × Rome Beauty)	0.05	7
Pyrus communis	0.20	10
Carica papaya	0.18	37
Scilla (in Liliaceae)	2.04	6
Datura stramonium	5.86	58
Oenothera organensis	6.50	55

to be added for *in vitro* growth depends on boron availability during pollen formation. Optimum concentration is generally between 5 and 150 mg boron per liter (*4*). Pear pollen from trees well fertilized with boron requires little or no additional amounts added externally to germinate in a sugar solution; pollen from trees or plants grown in low boron soils or not given boron sprays require the addition of boron at the higher levels. The requirement for pollen germination on the plant is usually fulfilled by boron in the style.

In 1933, Schmucker postulated that boron is involved in pollen tube growth. This hypothesis was recently confirmed with radioactive sugars and sugar alcohols that are used during growth to form pollen tubes (*8*). Boron modifies the incorporation of such compounds into pollen tube membranes. Presumably boron acts in conjunction with wall-synthesizing enzymes.

Calcium has been related to pectin synthesis in membranes. This cation also affects pollen growth and fertilization (*9*). The metabolic pathways involving boron and calcium are common to other types of growing plant organs. Few organs, however, grow as rapidly as pollen. Table 2 gives examples of rates of pollen tube growth. If trees could form cellulose and pectin as fast as some pollen tubes do, trees would grow about 100 feet a year.

RADIOACTIVE TRACER STUDIES

Radioisotopes provide a valuable research tool in pollen study. They can help determine the role of specific compounds in pollen growth. Contributions of pollen to the developing seed can be followed by using pollen with internal radioactive labels.

When flowers are removed before pollen development is completed, they will continue to develop if the stems are placed in water under proper growth conditions. These cut flowers will shed mature viable pollen. When a radioactive chemical metabolized by developing flowers is placed in the solution, it will move up the stem and enter the maturing flower. The developing pollen cells will incorporate some of the radioactivity. In this way, pollen with different radioactive labels, such as P^{32} or C^{14} can be prepared. Several combinations of labels and ways of using radioactive pollen are possible (*8*).

Tracer pollen techniques have reaffirmed the cytological evidence that generally only one male cell can fertilize the egg. However, other male cells or cytoplasmic elements in pollen grains may contribute to the developing seed. This finding was demonstrated by using nonradioactive pollen to fertilize the egg cell before radioactive pollen was added. It was then possible to determine if the radioactive pollen added after fertilization contributed anything to the embryo and developing seed. Swedish workers, using aspen trees in 1946 for the first such sequential experiments with radioactive pollen, found that one seed incorporated cytoplasmic material from seven pollen grains (*11*). By localizing the radioactivity in the seed, they could evaluate the contribution of a particular pollen to the offspring.

What influence the additional gamete has on progeny growth and whether the extra nuclear contribution can be carried over to successive generations is an active study area. This type of neo-Lamarkian research is being actively pursued in plant breeding institutes in Eastern Europe, including Russia. Radioactive labeled pollen and specific color genes in flowers and fruit are being used (*8*). Considerable disagreement exists between those institutes and researchers elsewhere on the importance of these nonnuclear cytoplasmic factors. It is still not generally agreed that these protoplasmic elements affect genetic expression, but certain conclusions can be accepted.

Genetically dissimilar and unrelated pollen can contribute to seed cytoplasm. Even though a foreign pollen cannot form viable seed, it may influence development of viable seed by one of several mechanisms. It may stimulate the female flower to secrete chemicals which keep the egg cell active, or it may stimulate an auxinlike compound in the flower. This compound may inhibit the formation of an abscission membrane across the stem. The flower, inhibited from abscissing, may finally be pollinated by a compatible pollen which can fertilize the egg cell. Or, foreign pollen may chemically activate the ovule to develop as though it were fertilized by a genetically acceptable male cell. Chemical stimulation of flowers by auxins to form seed was demonstrated by Gustafson more than 30 years ago at the University of Michigan. Many recent observations reported on labeled pollen can be interpreted in relation to such older studies. The

radioactive pollen allows measurement of growth and tissue interactions after pollination. This research "window" is helping to increase our understanding of fertilization and seed development.

INCOMPATIBILITY

Mechanisms of chemical interaction between female flower tissues and pollen are complex. Problems in this study area include incompatibility reactions. Such reactions occur after pollen lands on the stigma or equivalent female tissues. For example, after self-pollination, incompatible reactions which inhibit pollen growth commonly occur and result in failure to form seed. Genetic alleles have been postulated to explain observations in specific plant genera (*1*). In *Prunus avium* irradiation of maturing pollen modified the incompatibility system and increased seed formation (*5*). These incompatibility genes offer good working material for biochemical studies of cellular processes occurring in pollination (*6*).

Cytological investigations have shown that breakdowns in seed formation in certain pines and birches occur after cross-pollination but before fertilization (*8*). In such cases, the pollen may be incompatible with the female tissue of the other species of the same genus. In still other intrageneric crosses the pollen tube grows, but fertilization fails; or, the zygote formed after fertilization may abort before developing into a mature embryo.

Radiation, radioactive labeled compounds, and new microchemical analytical tools, such as a gel-acrylamide electrophoresis, are starting to provide our first clear understanding of incompatibility reaction mechanisms. Incompatibility reactions may involve several mechanisms and occur in different plant tissues (*6*). In fruit and forest trees we are just beginning to analyze breeding data and characterize the incompatibility systems which inhibit seed development (*1*).

PROBLEMS FOR FUTURE INVESTIGATION

CERTAIN research problems relating to pollen physiology are of particular interest to those concerned with fruit and forest tree production. Of the many factors which influence pollen germination, the effect of microclimate and cell nutrition on seed set are areas which must be studied more intensely. Problems related to low, erratic seed and fruit yield may be solved if we understand cell metabolism after pollination.

Studies in the 1930's showed that fungicides, such as Bordeaux spray, when used in orchards, often decreased fruit set. Investigators found that copper, mercury, or arsenic, as spray residues on flowers or brought in by bees, decreased pollen germination, resulting in low fruit set (*3*). Discreet use of pesticides and fungicides is essential for good crop yields. But these chemicals can modify pollen growth and thus affect orchard and tree production. Such potentially adverse effects require careful evaluation.

There are whole plant families about which little or nothing is known of pollination and fertilization. One such group, for example, includes the dwarf-mistletoes. These pathogens are a major economic pest of forest trees. Pollen of dwarfmistletoes has never been germinated in the laboratory. The life cycle of these plants from pollination to fertilization is still incompletely known.

The success of central seed banks has stimulated serious consideration of a question: Are pollen banks worth developing to assure genetic constancy and to expedite breeding programs? Many pollens are now stored and transported great distances to produce desirable hybrids. Would a central pollen bank stimulate research and facilitate agriculture? Dr. P. Maheshwari in India has suggested that a pollen bank would be essential to a worldwide plant breeding program. Possibly the United Nations Food and Agriculture Organization, or the forthcoming International Biological Program, will provide the opportunity to evaluate further the need for a world "Pollen Bank."
coming International Biological Year, will provide the opportunity to evaluate further the need for a world "Pollen Bank."

Pollen has been suggested as a dietary supplement. Certain pollens produce allergenic reactions in man, including hayfever. But some people eat pollen as a food supplement; it is sold as pollen candy in health food stores. What correlations exist in human physiology, if any, between the high estrogen content of date pollen and the use of date pollen food in ancient Egypt? Should nutritional laboratories investigate certain abundant pollens as potential human or animal food sources? Pollen as food would not materially affect food deficits in less-developed areas. But, some pollens may be ex-

ceptionally rich in limited natural products or vitamins. It might prove useful to recognize their potential nutritive value and possibly to include some pollens in common foods such as bread. More extensive use of pollen for a human diet supplement, however, has only very limited and questionable possibilities.

We must recognize that understanding how pollen develops, grows, and "sets" seed could provide knowledge essential to the control of seed and fruit formation. The pineapple industry has greatly benefited by the ability to induce flowering and pollination. Application of auxin sprays revolutionized and stabilized the economy of that industry in Hawaii. The agricultural industry would benefit considerably if fruitgrowers could respond to changed market conditions on a 4-month notice. Recognition of a heavy or poor crop in one area might help to effect considerable savings in another area with similar crops if production manipulation at the growing site were feasible.

Control of flower production and seed formation would be of even greater economic significance as a tool for the forest manager. Every ton of seeds a forest produces decreases by several tons the amount of cellulose a tree forms. A manager of a 15-year-old stand of trees being grown for pulp is not interested in flowers or seeds, since seed orchards can supply genetically known seed to replant the area when it is cut at age 20 or 30. In these circumstances inhibiting flower and pollen or seed formation is an economically desirable goal. A few chemical pollenocides, or gametocides as they are often called, have been developed for maize and row crops. In forestry and in tree crops, the potential of such a chemical provides a worthy goal for continued research.

The understanding of pollen physiology and pollen development and growth has outgrown the microscope stage. Pollen research has now moved into the center of a larger stage where it is being conducted by many related scientific disciplines. The biochemist is using radioisotopes, the cytologist the electron microscope. All investigations are probing deeper in the hope of increasing our understanding of pollen growth. Such knowledge can help the physiologist and field crop production specialist control and improve the uses of pollen. Meanwhile, pollen continues to function as it always has—fertilizing our rice, our wheat, our corn to yield food to feed our people. And it is pollen that gives rise to our table fruit and forest tree seed.

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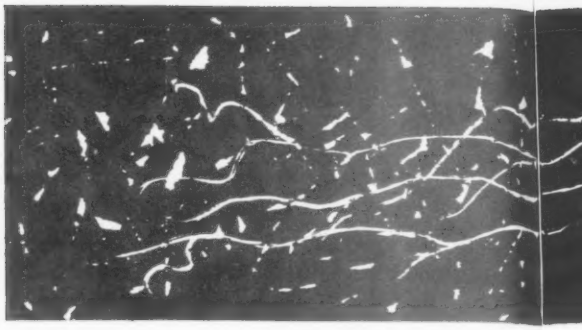


Arthur Wallace

Micronutrient Deficiencies in Plants

Many plant species grow poorly on calcareous soils because of failure to obtain sufficient iron. Iron deficiency of certain acid-loving plants occurs also in noncalcareous soils and also where heavy metal toxicities exist. An example of the latter has occurred in Florida where excessive amounts of copper have been applied to citrus and vegetable crops. Ten years ago, in contrast to other micronutrients, no effective means of supplying supplemental iron to plants was known. But now, synthetic chelating agents offer hope for correcting at least some iron deficiency.

Synthetic chelating agents are also proving quite useful in the application of zinc to fruit trees and to field crops. Zinc deficiency exists either where the soil contains insufficient zinc or where zinc is inactivated in the soil by fixation processes. Application of zinc salts easily corrects the first condition, but high levels are necessary to correct the latter, in which case zinc chelates are often advantageous. Manganese and copper chelates have also been used in plant nutrition but to a limited extent.

The iron chelate of ethylenediaminetetraacetic acid (EDTA) is useful in supplying iron to plants in noncalcareous soils. Iron chelates of ethylenediamedi-(o-hydroxyphenylacetic acid) (EDDHA) and of diethylenetriaminepentaacetic acid (DTPA) are useful in supplying iron to plants in calcareous soils. EDDHA is especially effective, and its higher cost is usually more than compensated for by the smaller amounts necessary to effect correction. Soil applications of zinc chelates of EDTA and nitrilotriacetic acid (NTA) are useful in supplying zinc to plants. Theoretically, the zinc chelate of hydroxyethyl ethylenediaminetetraacetic acid (HEEDTA) should be effective in supplying zinc but it has not been used.

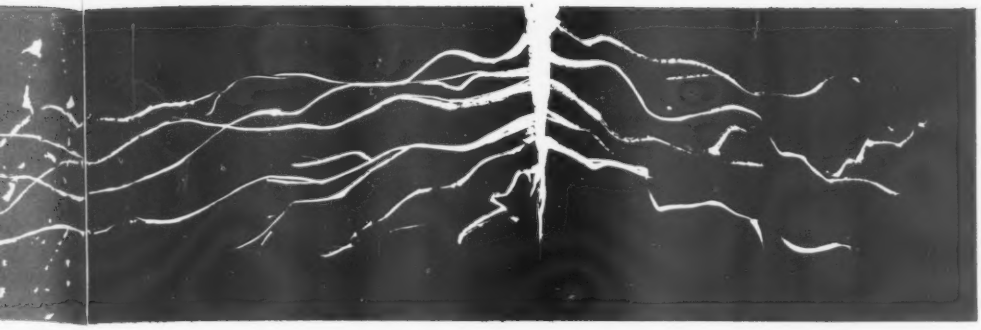
Chelating agents have been used to supply micronutrients to plants by foliar spray application as well as by soil application. At present it is doubtful if chelating agents offer any advantage over inorganic salts for foliar spray application, although there are some exceptions.

Plant Species Subject to Micronutrient Deficiencies

The great majority of soils contain large quantities of iron, but its solubility is so low that some plant species sometimes just do not get enough of it to meet their needs. Actually, it is not easy to understand how plants can obtain iron from soil. A better understanding of how they do would greatly facilitate attempts to solve iron deficiency problems in plants. Seldom does a true iron deficiency exist in soil, however, and the iron chlorosis commonly found in plants is usually "induced" by complicating factors.

In spite of the great insolubility of iron in soils, iron deficiency, or iron chlorosis as the disorder is commonly known, is peculiar to relatively few plant species. Several lists of susceptible plants have been prepared (*15*)[1] and growers usually try to avoid

[1] Italic numbers in parentheses refer to "Literature Cited," p. 24.



nts and their Correction with Chelates

such plants in production programs. The basic differences among plants that relate to susceptibility to iron deficiency are largely unknown. Jenny (*6*) has found that alfalfa plants can absorb insoluble soil iron by contact of hydrogen-saturated root surfaces with soil particles. He developed a hypothesis that after the iron became combined with the root surfaces, it could move into the plant via small negatively charged pores through which only cations can pass. Iron would precipitate in large pores of roots because of some of the anions simultaneously present. More information is needed on this hypothesis.

On the basis of Jenny's hypothesis, the nature of susceptibility to iron chlorosis would be either lack of production of H^+ at the root surface or insufficient narrow-charged pores in roots through which iron can pass. Other suggestions to explain susceptibility of species to iron chlorosis include a greater capacity to reduce ferric iron to ferrous iron at root surfaces of resistant species and varieties than at root surfaces of susceptible plants (*3*), the excretion by roots of substances that inhibit the uptake of iron (*14*), and the presence or absence of different types of terminal oxidase enzyme systems (*2*). An understanding of the pertinent factors most certainly would make it easier to correct iron chlorosis problems.

Most iron chlorosis is induced by environmental or chemical factors. The author has developed a hypothesis that all factors that induce iron chlorosis can be explained by a common phenomenon of competitive chelation (*12*). This means that (*a*) heavy metals induce iron chlorosis by competing with iron for metal binding sites in plant cells, or (*b*) miscellaneous organic and inorganic substances compete with binding sites in cells for available iron, or (*c*) that both processes are involved. In field-grown plants several complicating factors usually operate simultaneously to induce iron chlorosis, but they all quite possibly can fit into this unifying concept of competitive chelation. We need to know if they do and how. The complicating factors include low and high soil temperature, wet soil, bicarbonate in irrigation water, excess nitrogen and phosphorous fertilizer, excess copper, excess zinc, poor root growth for any reason, excess potassium, and sometimes direct sunlight. Calcium carbonate in the soil is the most common factor that induces iron chlorosis. Again, a full knowledge of the interrelations among these factors will help to devise means for solving iron chlorosis problems.

As with iron, some plant species are more susceptible to zinc and to other micronutrient deficiencies than are other species. Those susceptible to iron deficiency may or may not be susceptible to zinc deficiency. Little is known of the reasons for this. Trifoliate orange, which is used as a rootstock for citrus, is an example of a species subject to zinc deficiency (*7*). It is far more susceptible to both iron and zinc deficiencies than are other citrus rootstocks. Some varieties of corn, beans, and tomato

are also very susceptible to zinc deficiency. Some studies in the author's laboratory with trifoliate orange indicate a translocation failure within the plant. Information as to why this is so would be extremely helpful in at least designing experiments to learn how to control the deficiencies. Here, the use of chelation could be helpful.

The chain of reactions that results in failure of chlorophyll synthesis when iron or other micronutrient deficiencies occur is not known. Recent biochemical breakthroughs are making possible some preliminary studies to determine how iron deficiency interferes with protein synthesis. It has been known for some years that iron deficiency interferes in some manner with protein (enzyme) synthesis. At present, a fairly complete hypothesis for the nature of protein synthesis exists (*16*), but some parts of it are obscure.

This new knowledge of protein synthesis has made possible a few studies concerning the role of iron in protein synthesis. There is some evidence that iron deficiency results in failure of synthesis of a specific ribonucleic acid (RNA) (*11*). This ribonucleic acid in turn supposedly controls the synthesis of an enzyme or system of enzymes that results in chlorophyll synthesis. Additional information is needed to substantiate such a hypothesis, but it does provide a lead that may solve the physiological problems of lime-induced and other types of iron chlorosis.

Pehrur et al. (*8*) found that iron chlorosis interfered only with the protein of the chloroplast rather than with the cytoplasmic proteins. Possibly, there is a relationship between the chloroplast and the specific RNA that is not synthesized in iron deficiency. Knowledge of such a relationship may provide the necessary information to overcome such problems as the general failure of foliage sprays of iron to correct iron chlorosis in most plant species that are subject to iron chlorosis. This information also may help explain the induction of iron chlorosis by such factors as low and high soil temperature, excess heavy metals such as zinc and copper, excess lime in the soil, high soil nitrogen, high soil phosphate, and high soil bicarbonate levels. Indeed, iron chlorosis has been the most difficult micronutrient disorder to control. The primary reason is that we have so little basic information concerning it.

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Synthetic Chelating Agents in Plant Nutrition

In 1952 Stewart and Leonard successfully applied iron EDTA to correct iron chlorosis in Florida citrus trees (*9*). Since that time other useful synthetic chelating agents were developed for use in plant nutrition. Actually dozens of chemicals have been tested—including many naturally occurring compounds—but only a very few of the total have been adopted for practical use. The natural compounds, in general, are too susceptible to microbial decomposition for use in soil application, and therefore have more use in foliar sprays. Desirable characteristics of a synthetic chelating agent for soil application include (*a*) a degree of specificity for the metal being supplied, (*b*) resistance to microbial

decomposition, (*c*) stability of the metal chelate against hydrolysis and other means of inactivation in the soil, (*d*) water solubility of the metal chelate, and (*e*) availability of the metal from the metal chelate to the plant either at the plant root or within the plant after absorption (*13*).

The iron chelate of EDDHA meets the above characteristics admirably for use on calcareous soils. Its major drawback is that it is somewhat too expensive for the widespread use demanded of a universal means of correcting iron chlorosis. Chemical industry has done considerable research to decrease its production costs and to prepare similar derivatives that can be produced more economically. This program has been partially successful. Even with the cost handicap, iron EDDHA has many uses in the correction of iron chlorosis. For example, it is less costly to use iron EDDHA to correct iron chlorosis than to let a mature fruit tree die from the chlorosis. In general, however, metal chelates have a stigma of expense that discourages growers from using them. EDDHA has another drawback of some concern: It does not work very well for a few plant species. Reasons for this are not understood.

Iron EDDHA will supply iron to plants reasonably well on any soil, but iron EDTA will supply iron only on acid and neutral soils. Both are used, however, because EDTA is less costly than EDDHA; the choice depends on the type of soil. The differential behavior between the two relates to the stability of the iron chelates against hydrolysis in the soil. At pH values above 7, iron EDTA is easily hydrolyzed and the iron is precipitated as iron hydroxide. In this form it is poorly available to plants. At pH 7 and higher, many times as much iron EDDHA will remain in solution as iron EDTA. This characteristic of iron EDTA coupled with the fact that iron EDTA can be fixed on clay (*1, 13*) makes its use on alkaline and calcareous soils impossible.

The ability of zinc chelates to supply zinc when applied to the soil is due to the combination of characteristics of reasonable stability against hydrolysis and not too great a tendency for the zinc to be replaced by other metals available in the soil. Even though the chelating agents in use are more stable with iron than with zinc, iron already in the soil does not cause serious competition with zinc because of the tendency of iron to be precipitated as the hydroxide. Calcium can interfere with the utilization of zinc chelates. The zinc chelates in use are from 10,000 to 1 million times as stable against hydrolysis as are the respective calcium chelates. This allows a sufficient margin of safety for the zinc chelates. Application rates of from 0.2 to 2 pounds of zinc as chelate per acre will supply the zinc requirement on soils where inorganic zinc is readily inactivated. In the United States there perhaps are several million acres of cropland where zinc chelates can be used to improve yields. Appropriate zinc chelates may include some of the natural compounds; researchers in Nebraska have reported good success from some of them. Such compounds would have a cost advantage over synthetic chemicals.

The reasons that metal chelates are successful in supplying micronutrients to plants have been the subject of many studies. The results are not easily explainable. Synthetic chelating agents can be absorbed by plant roots and translocated to leaves. Some observations indicate that the chelating agents are absorbed by roots at a much lower rate than are metals supplied with them (*10*). This may suggest that the primary function of the chelating agents is to keep the appropriate metals available in the soil. The possibility exists, however, that an important function of the synthetic chelating agents is in facilitating the translocation of metals from roots to leaves in plants. Recent studies have indicated that EDDHA facilitates the translocation of iron in bush bean plants whether it is supplied before the iron, with the iron, or after the iron is applied (*5*). This could occur only if sufficient EDDHA were absorbed with the iron and translocated in plants. A large number of experimental results are available to show that chelating agents are absorbed and translocated by some species. The fact remains, however, that some plant species absorb chelating agents in very small quantities only.

Since susceptibility of some plant species to micronutrient deficiencies is related to ability, at least in part, to translocate metals from roots to shoots, it is highly probable that some of the benefit obtained from chelating agents with those species is with increased translocation.

Two important problems result from absorption

of synthetic chelating agents by plants. One concerns whether or not they are metabolized by plants; the other concerns the interactions chelating agents may have with the metabolic reactions in the plants.

If chelating agents are metabolized in plants, it appears to be very slowly. EDDHA appears to be slowly lost from soil and from plants following an application (*12*). This does not prevent it from being effective for several weeks, and a slow metabolism insures that little residue of the material remains. Actually EDDHA is exceptionally nontoxic to plants, and evidence shows it is not toxic to animals even in fairly large amounts. Of course, an extremely high level of it applied to animals without iron can compete with cells for micronutrients and cause injury. Incidentally, EDDHA has found some use in medicine. All iron chelates are easily decomposed when in solution and exposed to sunlight. Biological decomposition possibly occurs also to a limited extent.

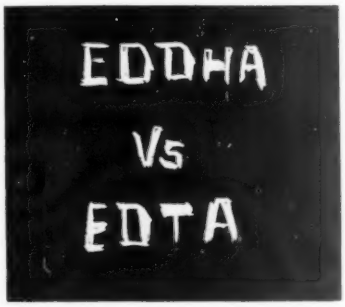
Synthetic chelating agents in plants conceivably can compete with cellular systems for available metals. Many of the systems in plants that bind iron, however, do so with a much greater stability constant than do any of the synthetic chelating agents. Plant cells certainly can compete with synthetic chelating agents for iron even when the iron is translocated to cells as the iron chelate. A large excess of chelating agent in a nutrient solution, however, can induce an iron deficiency in a plant by limiting the amount of iron absorption. Synthetic chelating agents, themselves, can induce micronutrient deficiencies in plants when used in excess (*12*). Manganese deficiency is a very common result of excessive application of iron chelates.

Interactions between metals and chelating agents emphasize the great importance of balance among metals in plant nutrition. One of the important functions of metal chelates seems to be that of improving micronutrient balance of plants (*12*). This is in addition to that of supplying metals. Toxic effects to plants of excess heavy metals can often be greatly lessened by applying synthetic chelating agents.

An important side effect of the use of chelating agents in plant nutrition is their effect on uptake by plants from the soil of radioactive fission products derived from the testing of atomic weapons. Some chelating agents do increase plant uptake of some fission products from soil. For example, DTPA has the ability to increase uptake of Y^{91} (*4*). Not enough of this agent is used nor is the isotope sufficiently abundant in soil for any great concern at the present time. Common sense dictates, however, that the effect be determined of any new synthetic chelating agent used in agriculture on the plant's uptake of radioactive fission products.

FUTURE RESEARCH

We know extremely little as to how plant roots are able to obtain micronutrients from soil. Without information of this kind, it is virtually impossible to solve problems of the differential ability of plant species and varieties to accumulate adequate quantities of micronutrients on certain soils. This is perhaps the most urgent research need if maximum use of micronutrients is to be achieved. We could use a large body of information on comparative mineral nutrition of plants, such as that developed for comparative biochemistry and comparative anatomy.



Another urgent research need relates to plant micronutrients and their interactions among themselves and the macronutrients. This information is urgently needed because of the tendency of growers to oversupply soils with micronutrients that have proved to be deficient. Overuse of zinc and copper has sometimes caused very serious complications. In other words, we need to learn how to correct a deficiency without inducing other problems. This specific research area has other ramifications. It concerns the reasons why some plant species fail to grow in acid soils, and it concerns the many factors that induce iron chlorosis. The need for synthetic chelating agents in plant nutrition could be greatly decreased if farmers themselves did not

induce iron chlorosis by mismanagement. Research on micronutrient interactions in plant species and varieties on different kinds of soils will help farmers to decrease the degree of mismanagement.

Some progress has been made toward an understanding of the nature of iron chlorosis, but more information would be extremely helpful. At least 15 different factors, either singly or in combination, are involved in the induction of iron chlorosis, but very little is known as to what happens to make iron nonfunctional in each case. Each soil and each crop often presents a different problem. Iron chlorosis is a complex problem and will remain as such until the factors that induce it are understood. Until then, its correction also will be just as troublesome.

Many sites of biochemical function of micronutrients have been identified. The mechanisms of metal function, however, are not generally known. This means that in many cases we know that a given micronutrient functions in a certain enzyme system, but we do not know how the micronutrient does its job. For the role of iron in chlorophyll synthesis, we do not even know what iron-requiring enzyme system is involved if any. Additional studies on the role of iron in nucleic acid synthesis are needed.

Synthetic chelating agents are becoming a popular means of correcting certain micronutrient deficiencies, but much research can yet be done on them. More new chemicals still need to be developed. Creativity and imagination on this question could produce perhaps hundreds of appropriate compounds. Such research is costly, however, and a less favorable balance than that which currently exists between population and food supplies will have to occur before much effort is expended on development of new chemicals.

Another need is for studies on the economics of use of synthetic chelating agents. Cheaper chemicals will increase use, but improved methods of application could be immediately applicable and thus partially solve the cost problem. Consideration should be given to irrigation application, banding, pelletization, coating, and seed application. In such research areas, it is of interest to note that the University of Idaho has developed a soil injection machine for applying iron chelates around the roots of trees. This is a step in the proper direction.

An all-out effort on foliar application methodology is perhaps the most urgent need in the practical solution of iron chlorosis problems. A successful foliar spray for iron would automatically solve much of the cost problem. The use of new chemicals and spreading and penetrating agents are some logical approaches to improved foliar application. New information on the permeability of plant cells would be exceptionally helpful in im-

proving foliar sprays. In any event, the improvement of foliar sprays remains as one of the great challenges of plant nutrition.

Some confusion still remains concerning the role of synthetic chelating agents in correcting micronutrient deficiencies. No attention has been given to reactions between root cells and synthetic chelating agents at root surfaces. Too little attention has been given to the differences in response of various plant species to metal chelates. Whether or not absorption of synthetic chelating agents by plants is necessary to correct micronutrient deficiencies is not known. Information as to whether plants can metabolize synthetic chelating agents is scarce; if they can, no information whatsoever is available concerning pathways of the metabolism. The problems concerning micronutrient deficiencies and their correction by synthetic chelating agents are indeed challenging ones for future research.

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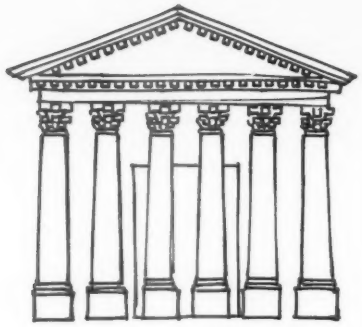
The Chronicle of Boll Weevil Research

Few insects have drawn so much attention from research workers in entomology as the boll weevil. From 1843 to 1960, approximately 1,500 different research reports were published by nearly 400 entomologists, each in his own way contributing to the vast body of knowledge about the control of a pest that for decades brought economic and social adversity to the Southern States.

Now, for the first time, these research reports have been abstracted and brought together in one volume for the benefit of entomologists and others working on cotton research problems. Titled "The Cotton Boll Weevil: Abstracts of Research Publications, 1843–1960," this annotated bibliography was prepared by Henry A. Dunn of the Cooperative State Research Service. Copies are available from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C., 20402.



FORUM

THE ROLE OF A SCIENTIST AS A COMMUNICATOR

To be a scientist, one must be a scholar—one who is knowledgeable, one who has an impelling desire to know.

The scientist is described as a special kind of scholar. He wants to know something new, to refine truth by defining in terms of his realities that which was unknown or obscured by doubt or superstition.

It is said that science in its purest form does not need immediate or pragmatic goals, nor does it need to be directed toward the social ends of survival or self-improvement. There is validity in this statement; yet much of science in recent times has become shaped in practical attitudes, becoming the bases first of invention, then of technology.

But whether the science is concerned about truth pursued by theoretical reasoning neutral on questions of utility, or the science perceives a practical objective, there are common characteristics. The work of the scientist—any scientist—is to discover the truth, formulate it, and make it a matter of public as well as professional knowledge.

The function of the scientist is to add to knowledge. He can add to knowledge only if he communicates to others what he has learned. Communication, then, is an essential, integral part of the apparatus of scholarship, whether the scholarship is devoted to unfettered discovery of truth or to application.

The fundamental communications of scientists are professional intercourse and dialog. This may be in the context of the university or research center where the organization is one of individuals in daily close communication with each other. Or it may be in the associations at a scientific meeting, a convened colloquium, a symposium, or an informal gathering of scientific peers and colleagues. It may be in the course of cooperative research or collaboration. Close associations present the finer opportunities to spread new ideas rapidly, to breed new discoveries, to correct erroneous observations and illogical notions, and to start the cross-fertilization of ideas. We would encourage and facilitate as we can such communication.

Scientific dialog, the writings and discourse that are its parts, is most important to the vitality and productivity of research. But its extension in the traditional scientific literature is the lifeblood of modern science.

Writings in the scientific literature form the bases for the widest extension of scientific knowledge. A scientific paper published in a scholarly and prestigious journal may be read by far fewer fellow scholars than each of us would like to believe. But an interested scholar here and there will take from the paper, perhaps refining the data and conclusions in his own laboratory and thought, and promulgate the written knowledge to many colleagues and students.

Publication in the scientific literature can be and generally is the most important product of the scientist. A scientist writing for publication in a scholarly journal will anticipate the scrutiny of his work by his peers. He will work and rework his statements of data and conclusion. He will become cognizant of the weaknesses and strengths of his schemes of attack and his methods of analyses. He will develop thereby his most creative and productive ability.

Publication of research in abstract, in progress reports of one kind or another, or in popularized form, or presentation of findings in a paper or talk at a scientific meeting or field day has real purpose and is encouraged. But these should rarely constitute ultimate publication. Only when the research is written so as to delineate fully the purpose or tested hypothesis, the important details of its conduct and analyses, and the considered conclusion, and only when written to meet the challenge of a scientific jury can a research truly be said to be completed.

Some staff members of experiment stations, unfortunately, are reticent to attempt publication in sci-

entific journals. Perhaps they fear criticism, or fear that the work will not measure up. Perhaps a previous attempt has brought editorial comment that has been less than kind. Editorial comment accompanying a rejected manuscript can seem harsh, discourteous, rude, insulting, and even downright ungentlemanlike.

Such is but part of the game. The rejected manuscript and presentation, in fact, may be faulty. New ideas are not easily accepted; statements that can be misunderstood will be misunderstood. The manuscript may well need revision to be comprehensible to men not familiar with the work, especially to those who must translate into another language. Or, in spite of long and arduous hours, the research results are repetitive or really unimportant and the conclusion can only be superficial or indefinite because the test was not critical enough.

If one has failed to get a manuscript accepted, and he remains a scientist, he must try again with new research or with new manuscript. To fail to take completed parts of research into the literature, to let important data lie in data books or remain presented and examined inadequately is to be wasteful of the resources entrusted to the scientist. Then, too, there is always triumph in publication of the once-rejected thought.

There is prestige, too, in publication in the scientific literature. Researchers live in a world where prestige is capital.

Henry Fielding, the inventor of the modern English novel, remarked in a digression, "It is not enough that your designs, nay, that your actions are intrinsically good; you must take care that they appear so." But gaining prestige is not an easy task. In the present atmosphere of scientific work in the United States, outstanding research requires a high intensity of effort. And a high intensity of effort and high quality of published writings are required to gain the prestige needed and to secure the support that can and must follow to build a strong career in research.

Part of the communications of scientists should be for a scientifically and technically trained, but not expert, audience. Professors and instructors can seek out what they need from the scientific literature, but those that do are usually expert. More often than not, instruction depends upon the textbook written by a scientist.

Colleagues in other disciplines may need to know scientific research in digest. Advances are necessary on numerous fronts to reach a new stage of agricultural science and technology. Scientists need to know the general state of development of related sciences in order to relate their work to the general progress or to select applicable techniques and methods they may derive from an associated science.

Basically, most of the official publications of the agricultural experiment station have their major usefulness for the inexpert, but scientifically literate audience. Textbooks, literature reviews, seminar presentations, science digests, journals, and the scientific press serve in more general areas to meet this fast-growing demand for communications by scientists.

Our function as a service agency will demand that scientists in colleges of agriculture continue and even increase their efforts to write for the audiences more directly dependent upon the body of scientific knowledge that such audiences, in turn, thus help to transmit. Fortunately, many scientists cordially and willingly meet the invitations to write, or do so on their own initiative.

But, I am sure that science will progress to an age of far greater specialization than we have yet imagined, making communication for the intermediate level of receptivity at once more imperative and more difficult. There is the belief (e.g., that of C. P. Snow) that as persons become more intensely educated, they have greater difficulty communicating with each other on the plane of their major intellectual concern.

The scientist has a major role, too, as a communicator to the general public. Writing or speaking for popular consumption has two major functions: putting research to work and establishing the image of science as an institution. In Scott Buchanan's words, if a scientist's concern is truth, it is his responsibility to be sure that science is used properly, that his science is not misused so that something false comes out of it. And, too, our institutions are public institutions. Whatever success we have will depend upon public awareness and support of our program.

A recent survey indicated that science stories in newspapers are as widely read as the sports page. Yet few newspapers have a full-time science writer on their staff, although we in agricultural sciences are somewhat more fortunate. Agricultural jour-

nalism has been a recognized course for study in its own right.

There are doubts, understandable doubts, among some scientists as to the propriety of having reporters report to the public. But, unless there are valid reasons for not doing so, it is best to cooperate with newsmen. If research is news, the story will be written with or without the scientist's help. And it is to the scientist's advantage to help the newsman get the facts straight, although in the process the scientist's ingenuity and patience may at times be challenged.

Many scientists do sense their responsibility to the public. In fact, many scientists increasingly believe that their special knowledge obligates them to translate this knowledge into terms useful to the layman in making decisions in his society. That such is proper is hardly arguable, but in so doing it is important for the scientists to appreciate how the amateur regards and respects the professional.

Laymen regard scientists with mixed and sometimes confused attitudes. They respect science, but it is commonplace to blame science and technology for such critical problems as overpopulation, domestic poverty in the midst of plenty, and agricultural surpluses. It is equally commonplace to assign to science and scientists, who are blamed for creating the pressing problems of human life, the responsibility for solving them.

It is proper and essential that the agricultural scientific community work toward informing the lay community about the nature and product of science. But the scientist must recognize that though truth is evident, confusions arise in testifying to the truth. We have ample example in this as various groups issue recommendations on the use of insecticides in Texas. Confusion or misunderstanding in presenting science not only results in confusion in the public mind but opens an institution of science to unwarranted denigration of its efforts by persons or institutions with competitive points of view.

The scientist should take due care that his opinions on matters outside of his competence not be perceived as the position of his science or his institution. For example, it is easy in times of political controversy for a member of a scientific community to speak his opinion, an opinion based on sketchy information or even on newspaper writings, and that opinion will be judged to be the consensus or policy of the scientific community. The public generally has no way of distinguishing casual statement from informed thought.

There is one additional role of a scientist as a communicator that requires some reexamination at this point. This is communication within the organizational structure. Though no direct line of communication is closed, generally communication within the college of agriculture flows through channels of organization. But it is imperative to collaborative and cooperative work that the barriers to interdepartmental communication be lowered as much as is humanly possible.

A major element of communication of scientists within an organizational structure is that in which the scientist applies for resource support of his program of research. This may range from an informal visit with the head of department to an organized project proposal to an agency of support. Here, too, the elements for successful communication must apply for all who ask, but especially for the younger scientist who has not yet built his place in the scientific community. Project proposals must be imaginative, well conceived, and well presented to gain the necessary attention in an atmosphere where the support is limited and already largely distributed.

I have spoken on the role of a scientist as a communicator. Some of you may find it more comfortable to be called a technologist, an inventor if you please, doing the highly important work of bridging science and technology. Your major interest may be in the systematic application of knowledge, creating a new plant line, or a new harvesting machine. Yet much of what is said of the scientist as a communicator must apply also to the inventor.

The search for knowledge of truth is an activity of man essential to everything else that is done. Each scientist must contribute to this search by his activities—by thinking, reading, working at his research, and by writing; by thoughtful and scholarly planning, competent execution, and effective communication in his research.

H. O. KUNKEL, *Associate Director*
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Texas A&M University

This Forum paper was adapted from a talk which Dr. Kunkel made at the Annual Staff Conference, Texas Agricultural Experiment Station, College Station, Tex., October 8, 1964.

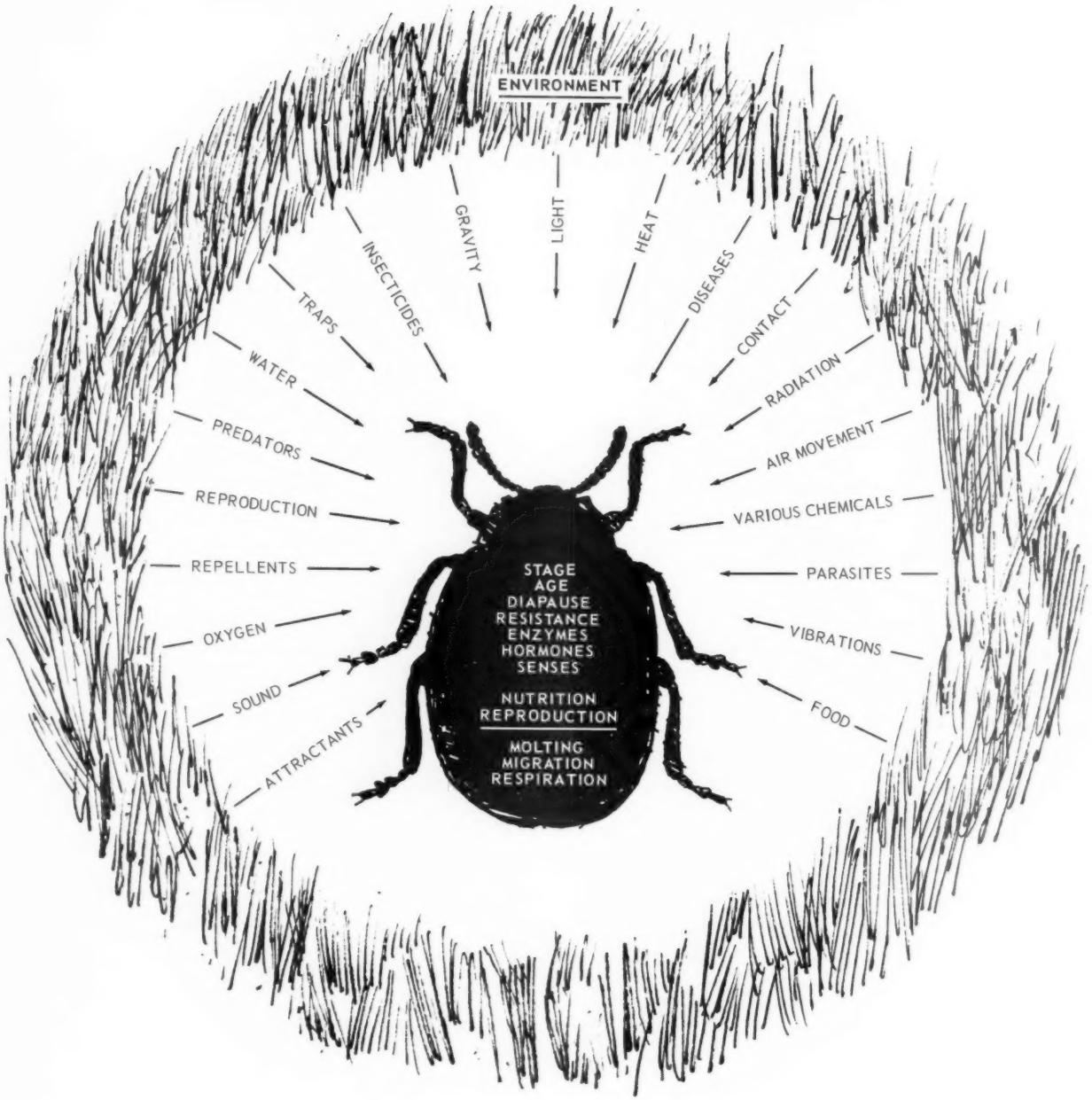


FIGURE 1.—Factors affecting insect activities.

APPROACHES TO INSECT CONTROL

E. R. McGovran

In the struggle for the world food supply, insects have defense in depth. Their widespread distribution and reproductive capacity make control by man difficult. This favorable situation for insects results from their great ability to adapt to the many environmental forces they encounter (fig. 1). History cites many instances in which insects successfully resisted man's attempts to annihilate them. Fortunately, though, history also records a limited number of victories for man—usually after much time, effort, and money have been expended. But still the struggle for the world's food supply goes on. Man continues his efforts to manipulate the factors in the environment; the interaction of these factors and the physiological processes determine the activities and survival of insects. A number of these factors and processes are shown in figure 1.

A most important factor in the environment is food. Under many circumstances the food supply of insects is sufficiently limited to prevent large population increases. However, intensive agriculture, with its typical concentration of cultivated crops or domestic animals in relatively small areas, has tended to set up an abundant and concentrated food supply for insects. Such conditions may lead to sharp population increases and agricultural losses.

Because of the importance of food as an environmental factor, one of the important avenues of improvement in insect control programs is that of changing the food supply. In making comparisons of varieties or strains of plants, studies have shown that insects feed upon some plants more readily than they do on others. On some plants, insects grow and reproduce more rapidly than they do on others. It is usually very difficult to find a plant immune to attack by a specific insect but which is still suitable for commercial use. However, it is often possible to find a plant that is both suitable for commercial use and is less attractive to an insect than other strains, or on which an insect will grow or reproduce at a slower rate. The use of a strain that will slow down the development of an insect pest—even though it may not eliminate it—would be of assistance in insect control.

Attractants that can be used to concentrate insect populations from a wide area where they may be trapped, killed, or sterilized are promising approaches to insect control. This method could materially reduce the size of the insect population in the affected area, and thus delay the rapid resurgence of a destructive population of pests. Another use of attractants is in bait sprays or dusts. Each spray droplet or dust particle would contain an insecticide plus an attractant or feeding stimulant. The insect in its natural search for food would be attracted to the dried droplet or dust particle, feed upon it, and be killed. With this method only a small fraction of the surface of plants needs to be covered by the insecticide. The eradication of the Mediterranean fruit fly from Florida was accomplished by this method. Much less insecticide was used than would have otherwise been needed.

Reducing the reproductive rate of insects is another approach to control that offers considerable promise. One technique is by sexual sterilization through irradiation. When the insects are reared in captivity, sterilized males are released and subsequently mate with wild females, resulting in sterile eggs. The spectacular eradication of the screwworm from Florida is a well-known example of this procedure. Another procedure is the use of chemosterilants. These, when used alone or combined with sex or feeding attractants, might be very effective in reducing the rate of reproduction. However, research on chemosterilants has not yet reached a point where such materials can be used safely in the field.

Detailed studies of the life and activity of insects that are pests or feed upon pests may be very rewarding in disclosing new approaches to insect control. A careful study of the different stages of the insect to determine the causes of mortality of the insects in the different stages in the field is often revealing. For example, the study of insect eggs laid on a crop to determine how many of the eggs were infertile, how many were eaten by predators, how many were killed by parasites, and other causes of egg mortality might point up the possi-

bility of a practical way to control pests by manipulating some environmental factor. If this same procedure were followed for each larval instar, the pupal, and adult stages, a complete picture of the factors that cause mortality in the population of the particular pest would be developed. Such studies, of course, would have to be done under field conditions where the actual crops were being produced. The effect of insecticides or other man-made means of insect control in such a study would probably be very striking. Studies of this nature have been called life-table studies of insects. On the basis of complete information of this type on several pests attacking a crop and the parasites and predators which feed on the pests, an overall plan of control could be developed on a sounder basis. The results of these studies would be more informative if they were correlated with various meteorological and agronomic factors. The results would probably indicate better approaches to insect control.

Living organisms that attack insect pests have been used extensively in the past to improve insect control. With presently improved methods of rearing insects on artificial media in the laboratory, it seems probable that use of laboratory-reared living organisms for release in controlling pests in the field will be greatly expanded. If adequate supplies of insect parasites, predators, or other parasitic organisms could be distributed over the infested area in the early stages of insect outbreaks, control pressure would be applied over as long a period of time as the parasitism continued to be effective.

Spiders have never been studied as extensively as other predators for the control of insect pests. This approach to insect pest control may offer opportunities. Possibly artificial rearing of spiders could be developed so that they could be released in the right numbers and at the right time to maintain the level of the insect pests below the level of commercial damage.

The use of insecticides for the control of insect pests has increased in efficiency to such an extent that in many instances it has become the main, if not the only, source of protection from insect damage. The main reason for this situation is that it is often difficult to predict in advance whether an insect pest population will become great enough to be destructive. However, the early stages of damage can usually be readily recognized. It is in this stage that some rapid-acting control must be applied to prevent more extensive damage. Such a situation is an emergency, and an insecticide application has usually been the best answer.

However, insecticides that are toxic to man and valuable animals or that persist in food, feed, soils, or water are undesirable. For these reasons, it is obvious that improved insecticides should be developed to replace those now in use that are hazardous or objectionable. In this respect, antimetabolites appear worthy of study. The development of insecticides that effectively control the pest and do not present a hazard from any angle is, of course, the objective of insecticide research.

A factor of major importance in the use of insecticides is the development of resistance by insects to insecticides. The same insecticide cannot be depended on to control a pest year after year. Resistance usually becomes evident as a rather minor decrease in the effectiveness of an insecticide. It is difficult to determine at this point if this is due to poor application, or some other factor reducing effectiveness, or to resistance of the insect to the insecticide. Often the efficiency in control goes down gradually as resistance increases and the real cause of the increased insect damage remains unknown. When resistance develops to a high degree, damage is severe and the ineffective insecticide is wasted. The importance of resistance in the control of insect damage is continually increasing. The discovery of the physiological or other processes that enable insects to become resistant to insecticides is a promising, although probably a long-time, approach to control of resistant insects. Once the cause of resistance of one insect to an insecticide is known, an avenue of attack to overcoming the resistance has been opened.

Integrated control is being developed for insect pests. In this, the biological and other factors that tend to control the pest are encouraged and insecticidal treatments are used only in a manner that will control the pest and have the least possible detrimental effect on the biological control of the pest. The selection of the kind of insecticide to use and the time, amount, and method of application all enter into this picture. This type of control can be extended to a great many of our insect pests. In fact, it seems desirable to develop integrated con-

trol programs in which all the factors that limit insect pest populations can be brought to bear. This would include not only parasites, predators, and disease organisms but also plant resistance, cultural practices, sterilization, and any other approach that can be developed to help hold insect pest populations at noneconomic levels. In such a program, a contribution of a small reduction in insect pest population in one place in the control program, when added to other small reductions in other places, could build up to a satisfactory control with a minimum of dependence on a "knockout" treatment at the beginning of severe damage by the pest.

Molting is a characteristic activity of insects which does not occur in most vertebrate animals. The physiology of molting, if thoroughly understood, might offer a new approach to insect control.

Diapause—the temporary stopping of growth or activity—is a condition which develops in many insects. Recent research has indicated that light plays an important part in the diapause in insects. By proper exposure to light and by controlling other factors, it is possible to affect diapause in some species. Diapause usually occurs to protect the insect during a period of adversity, such as the winter. If the development of diapause was inhibited, a suicidal emergence might occur when food was not available. This could effectively reduce populations of insect pests. Studies of diapause and its control present an interesting approach to insect control.

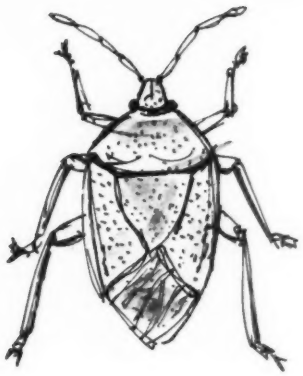
In the study of the reaction of insects to their environment, it is desirable to answer the following questions:

What environmental factors stimulate insects? For example, does the insect respond to light? Where are the light-receiving organs located on the insect? What is their structure? What physiological conditions in the insect enable it to respond to light in a given manner? What physiological changes in the insect are brought about by light? How can these be altered? Answers to these and other questions about all the environmental factors that stimulate insects would help to improve and open new vistas for insect control.

Thus, the possible approaches to insect control are as many and diversified (fig. 1) as (*a*) the various components of the environment that affect the insect, (*b*) the physiological processes within insects, and (*c*) the interaction of these two. Some of these have been discussed. Other approaches, as yet not clearly defined, may lead to further improvement in insect control. The ultimate objective—eradication of species in areas where they are pests—has been achieved in some areas by the application of new and old knowledge about insect activities and reactions. The possibilities of new horizons for insect control rest on more complete knowledge about insects and the application of this knowledge to their control.

The U.S. Department of Agriculture practices and encourages the use of those means of effective pest control which provide the least potential hazard to man and animals. This policy emphasizes the importance of continuing research in biological, ecological, and cultural methods of control. Moreover, entomologists are urged to exercise constant vigilance—not only in carrying out their research programs but also in directing others in the use of pesticides—to assure the protection of human health by avoiding unnecessary exposure of crops, livestock, fish, and wildlife.

An Example of Integrated Control of Insects:



STATUS OF THE SOUTHERN GREEN STINK BUG IN HAWAII

In the following article, an entomologist documents the account of Hawaii's efforts to eradicate a new pest from the State. His report, Review believes, is an outstanding example of the possibilities of integrated control in entomology as discussed in the preceding paper by E. R. McGovran. Moreover, it illustrates well the results that can accrue from wholehearted cooperation among agencies with related interests. Only other entomologists, no doubt, will be able to discern the intensive effort, the frustrations, and the satisfactions of able accomplishment that author Mitchell has implied in his commendable, low-key reporting.

On the morning of October 15, 1961, Choki Higa, truck crop foreman of the University Vegetable Crop Experiment Farm, noticed some odd-looking bugs feeding on beans, sweetpotatoes, and other crops in plots on the edge of the campus in Manoa Valley. Immediately he notified Frank Haramoto of the university's department of entomology. The pests were quickly identified as Southern green stink bug, *Nezara viridula* (L.). All stages of the life cycle (eggs, nymphs, and adults) were observed in the area, and from the size of the population it was assumed that at least two generations may have been produced prior to the discovery.

Identification of the stink bug caused understandable alarm, because it is a serious pest of many fruit, nut, and ornamental plants in the Southern United States and many of the countries and islands of the Pacific Basin.

Immediately, entomologists from the State department of agriculture, Hawaii Sugar Planters' Association, Pineapple Research Institute, and the University of Hawaii met and collaborated in making surveys and attempting to eradicate the infestation. Federal and State plant quarantine inspectors were alerted to the presence of the pest. A stopgap program of spraying all infested areas with malathion was initiated.

Surveys in October and November located infestations at the McKinley High School garden (2 miles from the university), upper Manoa Valley and Kaimuki (4 miles from the university). In December of the same year, extensive and widespread infestations were located at Ewa, Waipahu, and the vegetable-growing areas in Makaha Valley and Lualualei Valley of the Waianae district (40 miles from the university). *Once the widespread infestations were discovered, all attempts toward eradication ceased and emphasis was placed on biological and chemical control studies of the pest and quarantine inspections to prevent further spread to the neighbor islands.*

The stink bug continued its spread over Oahu in 1962. It moved eastward to Waialae-Kahala (5 miles from the university) by April; Aina Haina and Waimanalo (13 miles) by June; to Kaneohe Bay by November; westward to Aiea (10 miles) in May; and Pearl City (20 miles) by June.

Nezara encircled the island of Oahu in 1963 when it spread to Wahiawa, Haleiwa, and Waialua by May; Kawailoa, Waialee, Kahuku, and Kahana by June; Hauula in July; and Laie in October. The stink bug has been found on Oahu from sea level up to 1,265 feet elevation.

Kauai

It was nearly a year after its discovery in the State before the pest was collected on a neighbor island. In August 1962, an infestation was confirmed on the island of Kauai located 90 miles north of Honolulu. The stink bug is now widespread over the island.

Hawaii

The second neighbor island to be infested was the big island of Hawaii located 250 miles south of Honolulu. The first infestation was reported and confirmed at the port of Kawaihae on the northeast coast of Hawaii on June 13, 1963. An alert citizen noticed the strange insect on the window, captured it, and called the State department of agriculture entomologist in Hilo who recognized it. The infestation appeared to be localized and the State department of agriculture and University of Hawaii personnel initiated an eradication program. On June 29 a single specimen was found near the waterfront area in Hilo on the opposite side of the island. Surveys of the immediate vicinity at Hilo and Kawaihae failed to produce other infestations. Surveys did locate several large infestations along the Kailua-Hona coastal road to Keauhou Bay on July 2 and 11. Insecticidal applications of malathion were made to all infestations and surveys were intensified in the area. On July 18 an extensive, active infestation was found between the coastal road and the mauka belt highway where the terrain is practically inaccessible for ground equipment, and eradication was considered economically unfeasible. This infestation was in cattle-ranch land on weed hosts beneath the tree canopy and was inaccessible to aerial application. *With such widespread infestations, the eradication program on Hawaii was discontinued and plans for the mass production and distribution of parasites were started by the State department of agriculture.*

The stink bug moved south to Hookena on the Kona side by July 18, 1963, and around to Pahala in February 1964. It has also spread from Honokaa (February 1964) south to Hilo and below Hilo to Keeau, where the largest macadamia nut orchard (1,500 acres) is located. The stink bug has been found from sea level up to 1,900 feet (at Kainaliu), and one infestation in Chinese cabbage was reported at 2,650 feet elevation at the Laumilo Experiment Station, Kamuela, in September 1964.

Molokai, Maui, and Lanai

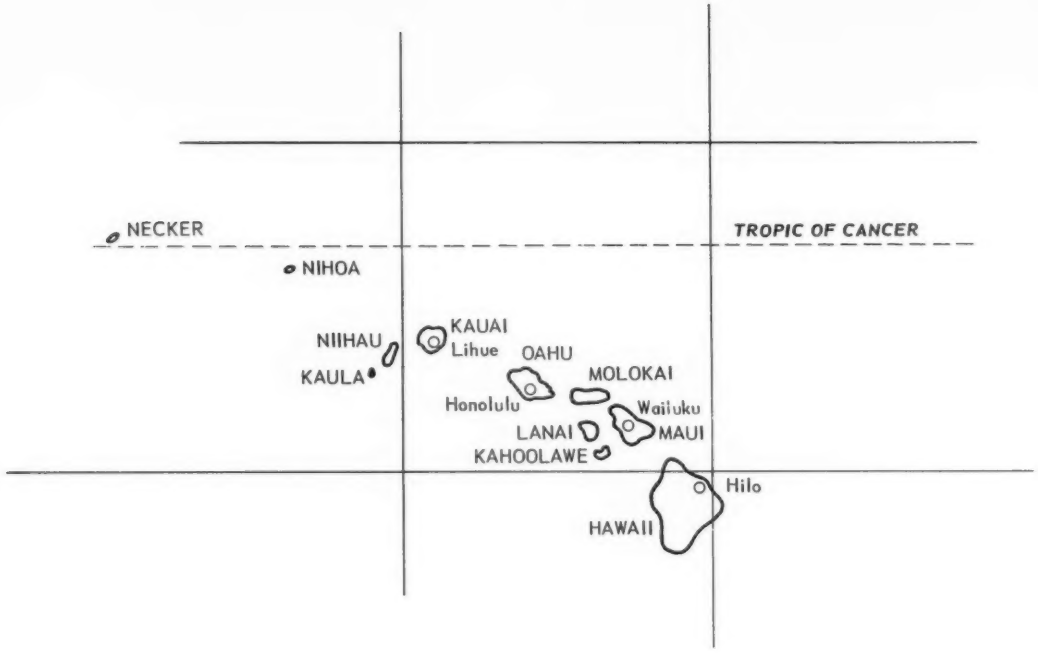
The first infestation on Molokai was found at Kaunakakai on August 25, 1963. Now the stink bug is spread over the island from sea level up to 878 feet elevation.

Four separate infestations were located on Maui at Wailuku, Kahului, Paia, and Lahaina on August 26–29, 1963. The stink bug is widespread over the island and can be found from sea level to 1,700 feet elevation.

The first infestation of the stink bug on Lanai was found on August 27, 1963, at Lanai City opposite the cargo ramp where all incoming produce is deposited for pickup by the people of Lanai. A second infestation was found at the harbor area of Kaunalapau. The pest can be found from sea level up to 1,632 feet elevation.

The insect has not been reported from Niihau, the privately owned island off the coast of Kauai, and the uninhabited island of Kahoolawe located between Lanai and Maui.

Infestations on all islands followed a regular pattern of increasing in numbers on the weed hosts in uncultivated areas and then moving into cultivated crops or gardens when the weeds dried up or because of population pressure and lack of suitable food. The more important weed hosts in Hawaii are: Spider Weed (Capparidaceae) *Gynandropsis gynandra* (L.), Spiny Amaranth (Amaranthaceae) *Amaranthus spinosus* (L.), Asystacia (Acanthaceae) *Asystacia coromandeliana* Nees., Popolo Berry (Solanaceae) *Solanum nigrum* (nodiflorum) (L.), Castor Bean (Euphorbiaceae) *Ricinus Communis* (L.), Cheese Weed (Malvaceae) *Malva rotundifolia* (L.).



Economic Importance

The stink bug is active throughout the year in Hawaii. Females lay their eggs on the undersides of leaves and each egg mass averages 70–80 eggs that hatch within 5–8 days. Nymphs molt five times before reaching the adult stage. The first nymphal instar is gregarious and does not feed but may ingest water. Size increases with each successive molt. It takes from 35 to 45 days for development from egg to adult. Adults mate 7 days after emergence and egg deposition begins 14 days later.

Damage to plants is caused by the feeding of the second to fifth nymphal instars and adults. The insect prefers the young, succulent, rapidly growing tissues found in buds, blossoms, and fruits. Feeding sites in fruit are usually localized and characterized by dry, cottony pockets beneath the skin. The point of penetration of the mouthparts is not visible to the unaided eye, but is large enough for micro-organisms to enter which may cause rotting of the fruit.

Heaviest losses to the cut flower and foliage industry occurred on Oahu in the spring and summer of 1963. In some areas growers had complete loss of blooms on hibiscus, vanda, dendrobium, and cattleya orchids. Only two growers have experienced any loss in 1964. Feeding of the stink bug caused the buds to shrivel, dry up, and drop.

The stink bug has caused damage to many fruit and vegetable crops. Feeding occurred on soybean, string beans, yard long beans, tomatoes, corn, watercress, guavas, mangoes, litchi, citrus, acerola, and others. Reports of damage began in the summer of 1962 and increased to a peak in 1963. Some home gardeners and part-time farmers on Oahu experienced total losses of their fruits and vegetables in 1963. Only one report of loss on mangoes was received in 1964, but the stink bug is still present in the vegetable gardens on beans and tomatoes.

The macadamia nut industry on Oahu has been hit hard by the pest. A stink bug can penetrate through the husk and the hard shell of a macadamia nut to reach the kernel in less than 5 minutes. It will attack the nut in any stage of development, thus causing economic loss to the grower. The kernel spotting, similar to that occurring on pecans in the Southern United States, was first noted on October 8, 1962, in harvested nuts from trees located in Nuuanu Valley, Oahu. Additional reports were received from Waipahu and Ewa. The harvest season on Oahu extends from July through

January. In 1963 stink bug feeding damage to macadamia in four orchards on Oahu ranged from less than 1 percent to 72 percent. In 1964 the damage decreased to 20 percent. On the island of Hawaii about 1 percent damage was reported in the fall of 1963 at Kona. In 1964 the same area had 0.7 percent in January, 5.7 percent in February, and 13 percent in March. One farmer reported 98.6 percent loss of his crop in 42 acres of macadamia in June of 1964.

The stink bug has been observed feeding on green and ripe coffee cherries on the island of Oahu, but has not become a problem in the major producing areas in Kona, Hawaii.

Infestations on various weed hosts have been observed in pineapple and sugarcane fields, but the stink bug has not damaged either of these crops.

Stink Bug Control

Control of the stink bug has been attempted and investigated along two general lines, biological and chemical. All entomologists in the State have been cooperating and uniting their efforts to combat this pest.

A number of parasites have been introduced into Hawaii by the State entomologist, State department of agriculture. On February 6, 1962, adults of a small Scelionid wasp, *Telenomus basalis* Wollaston, received from Australia, were released in Moiliili, Oahu. This insect is a stink bug egg parasite and has become widespread over Oahu. Field collected stink bug egg masses are either unparasitized or heavily (90–100 percent) parasitized. Davis[1] reported an average parasitism of 94.9 percent in 10 egg masses collected in the field the last six months of 1963. Parasitism by the egg parasite remained high in 1964. The egg parasite has been released on all other stink-bug-infested islands and has become established on Oahu, Maui, and Hawaii. Two other egg parasites, Encyrtid wasps, were received from Trinidad and released on September 24 and October 1, 1962, but have not been recovered to date.

The adult stink bug parasite, *Trichopoda pennipes var. pilipes,* a beautiful Tachinid fly, was received from Trinidad and released by the State entomologist at Makaha Valley, Oahu, on April 2, 1962. It is now widespread over Oahu and has been showing from 10 to 80 percent parasitism of field collected bugs. During the last six months of 1963, 40.9 percent of the field collected stink bugs had eggs of Trichopoda deposited on them. In 1964, 44.5 percent of the field collected bugs carried eggs of the tachinid fly. Adults of the tachinid fly are commonly seen in the field where stink bugs are found. The female parasite literally dive bombs on the adult stink bugs and lays her eggs on the body surface of the victim. Although many eggs are deposited on the stink bug, only one will develop into a parasite and kill the host. This parasite has been released on all infested islands and is established on Oahu, Kauai, Hawaii, and Maui. A shipment of these parasites has been received from Florida and will be released on the infested islands. They should be more effective in the cooler areas than the Trinidad strain. Entomologists of the State department of agriculture and the University of Hawaii are following the spread and establishment of these parasites closely.

Most commercial growers with a regular insecticide spray program have been able to control stink bug infestation in their crops. Malathion, diazinon, dimethoate, Zectran, naled, carbaryl, endosulfan, Bidrin, and SD9129 in experimental field trials have been effective in controlling the pest. DDT was ineffective in Hawaii. Most of the insecticides have been effective as contact poisons, but of little value in controlling migrating populations. Those materials that have systemic qualities were toxic to the younger instars for a period of time. Data from these experiments aided in obtaining USDA approval of malathion in July 1964 and endosulfan in December 1964 for use on macadamia nut orchards.

The stink bug has decreased to low levels on Oahu because of the activity of the parasites and the wise use of insecticides. It is still a menace on the neighbor islands of Hawaii, Maui, Molokai, and Lanai. But entomologists of the State department of agriculture, U.S. Department of Agriculture, and the University of Hawaii are continuing their fight against this pest.

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[1] Davis, C. J. 1964. The introduction, propagation, liberation and establishment of parasites to control *Nezara viridula* var. *smaragdula* (Fabr.) in Hawaii. Proceedings of the Hawaiian Entomological Society 18(3):369–375.

The Use of Biometric Methods in Entomology

F. M. Wadley

The use of biometrics in entomology serves, in general, the same purpose as in other branches of experimental science—an application to problems of sampling and experimentation. We seek assurance that our decisions from samples or experiments are fairly reproducible—that others who may repeat our work will not surprise us by reporting widely different results. We base our work on known mathematical behavior of successive determinations of the same thing. This approach, of course, requires several repetitions or "replications" of our test. Armed with the numerical results from these, we can estimate how far our sample result is from the true mean, and within what limits the true mean is likely to lie. But we also need an idea of what variation future repetitions are likely to show. In such situations, we have an elaborate body of methods of mathematical analysis, which has been developed for providing the needed information.

In looking over statements in the older literature of entomology, we can find many instances of confusion, disputes, and setbacks caused by lack of replication. Fortunately, some of the shrewder workers recognized intuitively the uncertainty of experimental results and were cautious in drawing conclusions.

There are at least three kinds of statisticians—sometimes hardly conscious of each other's existence. The first and most numerous class does not deal with analysis of experimental results, but merely with tabulation, summation, and sometimes with study of trends. This is the type of work the public usually thinks of as being statistical. The Civil Service Commission formerly recognized these workers as "survey statisticians." The second type deals with analysis of experimental results and with planning experiments. The Civil Service Commission formerly classified such workers as "analytical statisticians." The third type—"mathematical statisticians"—do not deal much with arithmetic or with practical experimental results, but are occupied in developing new statistical methods or modifying old ones. Their work utilizes algebra, calculus, and higher mathematics.

Some special considerations apply to standard methods of statistical analysis in entomological problems. These methods as originally developed are suited to use with *measurements*, which can be made as fine as desired. If a foot is too coarse a unit, measurements can be made in inches or millimeters. In entomology many results are in the form of *counts* of indivisible units. Their statistical behavior is somewhat different from that of measurements, and the methods may require modifications. Not all entomological data, however, are counts or enumerations; experiments may require such measurements as spray deposits or crop yields But in typical work we are interested in the density of an insect population or in the proportion or percentage

Based on a talk given before the Entomological Society of Washington, D.C., Oct. 1, 1964.

of a population affected by some practice. Both approaches are based on counts.

When counts are based on fairly large numbers, not varying widely from one unit to another, the standard methods can be used with a satisfactory degree of accuracy. When numbers are scanty, or where very large and very small numbers are mixed together, it may be better to *transform* the counts. Square roots, logarithms, or some other suitable function of the counts can be used in analyzing such cases. The decisions based on such analyses are likely to be more accurate than they would be from analysis of untransformed counts, because of better distribution and other mathematical considerations.

The application of methods calls for close cooperation between statistician and experimenter. Cut-and-dried transmission of papers without personal contact seems unsatisfactory. In contrast, personal contact creates a healthy condition in which experimenter and statistician can rejoice together over a favorable outcome. Obviously, the statistician must understand experimental objectives and methods. It is desirable for interested experimenters to carry out their own analyses, with proper review, because such analyses tend to improve future research. The great R. A. Fisher has said that applying statistical principles may be as important as working them out. Some harried experimenters and analytical statisticians may wish that some mathematicians and mathematical statisticians understood this precept better.

In the actual work of analysis there are three basic procedures. First is the calculation of variance and its related measures in cases of simple variation. The deviations of the n individual cases in a series from their mean are squared and the squares are summed. When this sum is divided by $(n-1)$, the quotient is the variance; its square root is the standard deviation. The standard error of the mean is estimated by dividing the standard deviation by the square root of n. The meaning and use of these measures must be grasped by the student. The calculation of sums of squares of deviations is a large part of the actual labor of biometric calculations.

Second is the subdivision of variances due to various causes—known as the analysis of variance. In practice, short-cut formulae are used, and it is the sum of squares of deviations from the mean which is subdivided. The variances are then derived from the parts of the sum of squares. An experimental situation with simple variations is uncommon. Nearly always there are several causes of variation, and the variance ascribed to each cause must be estimated. The use of analysis of variance has facilitated development of experimental plans.

Third is the study of relations between two or more variables. This involves regression methods, and uses sums of products of deviations from the mean, as well as sums of squares. It may be noted that sums of squares of deviations are always positive, but sums of products of deviations may often be negative.

These three techniques are basic in biometric analysis, and with amplifications or combinations are used in solving all sorts of problems. [1]

Entomological practice has contributed to biometric thought in several ways. As previously noted, insect counts are often transformed for more efficient analysis. The commonest transformations are logarithm, square root, and "angle," but others are used. They have general application to biological counts, but have received special attention in entomology, because of the importance of insects and the large amount of counting work in insect study projects. Here, insect study has given an opportunity for development of general principles. In the same way the distribution of populations and of counts per unit (such as numbers per plant or per square foot) has received special study in entomology and is of general application in biology. The mathematical form of distribution—the proportion of units having zeros, ones, etc.—may lead to useful ideas as to how the population got there. A third subject which has developed largely in entomology, and which applies to biology in general, is that of *dosage-mortality*. The percentage of mortality as it increases with increasing dose of a poison is studied by modified regression methods. The importance of such procedures in studies of insect control is obvious. So much has been done in developing methods that dosage-mortality constitutes a special branch of

[1] For further study, see one of the numerous statistical textbooks, such as that by G. W. Snedecor, 1956, *Statistical Methods* (5th ed.), Iowa State College Press.

biometrics.

Entomologists are always interested in sampling methods, since decisions about uncounted millions of individuals must be made from examination of a few hundred or a few thousand. Biometric studies of sampling, as well as mechanical methods of managing sampling, are subjects too broad to receive more than mention here. It may be noted, however, that a large number of small groups of units is always better than a few large groups. For example, 20 apples from each of 10 trees gives a better sample than 100 apples from each of 2 trees. There are, of course, practical limits to this spreading of sampling.

Since insect populations in entomological field plots are usually estimated by sampling, we typically may have a replicated experiment with sampling within the experimental units. Thus, the replicated plots are themselves, in a sense, samples of the conditions tested, and therefore we have samples within samples. The plots, not the within-plot sampling, must be the basis for error estimates.

Some years ago a research worker proposed to test the effect of some cultural practices on orchard insect populations. The scheme called for treating one orchard and leaving another untreated as a check. The proponent of the scheme was quite concerned about sampling methods to estimate population in this unreplicated experiment. But it should have been obvious that the two orchards probably differed irrespective of treatment; and it would be difficult to tell whether the difference was due to the treatment, to the original condition of the orchard, or to both. Obviously, to get conclusions of value there should be several units (in this case orchards) both treated and untreated. In such cases, when the whole experiment is almost meaningless, a biometrician will suffer considerable anguish when he is asked to suggest sampling methods or to make calculations from sampling results.

Some entomologists, unfortunately, seem to be less interested in experimental design than in sampling. In planning experiments, all previous knowledge should be utilized. Questions for test should be clearly defined, and the plan should be arranged to give the questions a definite answer. When the answer is forthcoming it should be accepted. Modifying a clear decision to fit previous opinions should be avoided. Independent replications of a test will provide a vital estimate of error, or of the amount of variation found in trying the same thing over again. Arrangement of tests for close comparisons will add to efficiency. Such objective approaches are much better than the old practice of trying something new, forming an opinion by observation of unreplicated data, and maintaining the opinion against all comers. The latter approach has led to much confusion in the past.

Abbreviations in Journal Citations

A comprehensive index, identifying scientific journals from the abbreviated title citations found in scientific references, is being compiled by the University of Rochester (N.Y.) Library. The index will serve as a basis for determining the minimum acceptable amount of information required in an abbreviated scientific journal title citation to identify each journal as a unique entity.

Preliminary to determining the acceptable minimum for an abbreviated citation, actual citations are being collected, searched, tabulated, and cross referenced.

Since the serial lists contain entries for more than 250,000 separate titles, obviously one cannot expect to pinpoint a single scientific journal from ambiguous citations. It has not been surprising to find that a major proportion of abbreviated scientific journal citations are so vague that the citation is equally applicable to several different journals. The greatest number of possibly applicable pertinent journals found for a single citation is 22.

Additional collections of abbreviated citations are being made by library staff members, and it is expected that these will make a full index indicating all possible journals to which any existing citation may refer. A study of this material will provide a basis for determining the acceptable minimum for proper identification.

From *Current Research and Development in Scientific Documentation No. 13*, National Science Foundation.

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REVIEWING A TECHNICAL PAPER

IN the sequence of events progressing from the actual research project to the published report, few steps carry as much importance as that of the prepublication, or technical review. Its purpose, of course, is to assure technical papers of high scientific standards. Without adequate and effective review, the world's body of scientific knowledge would be thoroughly unreliable, to say nothing of being so voluminous that it would overwhelm our documentation facilities.

Reviewing is a costly procedure, because it often requires the services of top-level staff members. For this reason, the reviewing task should be accomplished as quickly and efficiently as possible. Unfortunately, good reviewers are difficult to find. Too often, they approach the job without really knowing what they are supposed to do. This approach not only produces some unfair and inaccurate reviews, but also wastes time of busy people.

The following guidelines constitute a sound procedure for effective critical review of a technical paper. Developed by the editorial staff of *Agricultural Science Review,* these guidelines, if followed, should contribute much to the smoother functioning of the author-reviewer partnership and, consequently, to the strengthening of research management.

On the first reading, a reviewer should determine if the paper is worth publishing. The worthy paper may contribute substantially to the body of scientific theory; it may contain numerical data that will stand the test of time; it may show ways to improve established methodology, or, it may offer solutions to problems of fact, problems of value, or problems of means.

In your role as a reviewer, try to decide if the paper meets one of these criteria. If you cannot determine this on the first reading, put the paper aside for a few days and then read it again. If you decide that the paper does not meet one of these criteria, it should be rejected at once. But don't neglect professional courtesy; tell the author why the paper is unacceptable. Perhaps it can be saved by rewriting. Give the author the benefit of your knowledge and understanding of the subject. Be explicit. Talk things over in person if possible. Don't waste your time pointing out minor errors in the paper if it needs a major rewrite.

You may decide that the paper is not worth publishing no matter how many times it is rewritten. Again, it is your duty to tell the author why.

If you decide that the paper does meet one of the criteria, read it again to become thoroughly familiar with its contents. Make sure the author has clearly stated the purpose and scope of the research. Has he limited the literature cited only to that which is pertinent? Because of your knowledge of the subject matter, you may discover that the author has overlooked other research that will alter his interpretation of his own data.

Examine the author's basic data carefully. Has he exploited the data fully? Perhaps he has overlooked some important points that need to be emphasized. Your review of the author's conclusions demands closer scrutiny than that of any other section in the paper. See if you can determine if his conclusions are valid and adequately supported by the data in the paper. Has he properly oriented his conclusions to practice?

Next, look at the policy implications of the paper. Is the topic handled in such a way that will cause needless embarrassment or difficulties to the institution sponsoring the research? If you spot problems of this kind, it is your duty to explain tactfully to the author how the offending sections should be handled.

If you are conscientious in your reviewing assignment up to this point, you will discover that it takes time and makes you think. This is good, because it shows you are performing your greatest service for the author. He needs all the technical advice and help you can give him. You may have the opportunity to point out minor errors in logic, to bolster the author's interpretation, or to suggest new interpretations of his data.

Any comments that you want to pass along to the author in this reading should be written on a separate sheet of paper, not on the manuscript. Key your comments to the page and paragraph numbers. Nothing is so exasperating to an author as an unexplained question mark or a facetious comment written on the margin of the paper.

U.S. GOVERNMENT PRINTING OFFICE : 1965 O—764-444

At this stage you should also consider whether the subject is presented in the most suitable form and manner for the intended audience. If the author has not indicated where he intends to publish the paper, he has violated one of the cardinal principles of technical writing. If the paper does not fit the proposed audience, you might suggest ways of adapting it; or suggest a new outlet.

Your function as a reviewer will be complete at this point if the manuscript will receive critical editing after it leaves your hands. However, if the manuscript will receive only cursory editing, then your job as a reviewer is not finished.

Put the manuscript aside to allow the trees to recede into the forest. At this last reading, consider the manner of presentation. If you can assume the attitude and approach of a reader, your evaluation will become more meaningful. Examine the structure, the arrangement of ideas, the relation of hypotheses and experimental tests. Are the tabular data hopelessly jumbled? Is the art work functional and correctly prepared? Does the author's message reflect clarity and precision in writing so that the reader can readily grasp the message? Actually, your evaluation at this reading should not be difficult if you have assured yourself that you know what the author is saying.

At no time during the reviewing process is it your duty to red pencil the grammar, the syntax, the punctuation. Features of rhetoric and literary quality, though within the scope of comment of any reviewer, can best be handled by skilled editors. Although there is nothing wrong with commenting on the rhetorical features of the manuscript, the detailed editing is not in your realm.

Finally, as one scientist has said, "Reviewers, like judges, are sometimes biased, careless, and even downright wrong." The reviewing process is one that demands good judgment, common sense, and a charitable attitude toward the fellow on the other side of the fence.

AGRICULTURAL SCIENCE REVIEW is a critical review journal designed to provide a common point of understanding among agricultural scientists by publishing authoritative commentary on the current state of agricultural research. *Review* does not publish primary research reports. Yearly subscription rate is $1.25, domestic; $1.50, foreign. Single copies are 35 cents. Subscription orders should be sent to the Superintendent of Documents, Government Printing Office, Washington, D.C. 20402.